

Another leap forward in Japan

Japan, notoriously neglectful of its universities, now seems bent on rescuing them with a substantial infusion of yen for rebuilding from the education ministry.

Who says that Japan knows better than anybody what the market wants, but is devoid of creativity? Most people, including many Japanese. They may not have heard that the Japanese education ministry (Monbusho) hopes to spend \$110 million a year (beginning next April) on the renovation of Japan's 96 universities (see *Nature* **353**, 292; 26 September 1991). Much of the money will go to science and engineering departments. The result should be a decisive modernization of Japan's basic science. What does this mean?

Those who wish to understand Japan, and its self-contradictions, must first grasp a simple truth: the government of the now-richest country in the world is up to its neck in debt. The explanation is twofold. In the heyday of growth in the 1960s and early 1970s, the government overborrowed to build expensive things such as bullet train lines, and the Japanese people are now reluctant to foot the bill by paying higher taxes.

Traditionally, they have instead put up with unreasonably low interest rates from the commercial banks, but that means of dealing with a badly balanced budget has been foreclosed by US insistence that Japan's financial markets be deregulated. Two years ago, there was a near-revolution because the government levied a sales tax of 3 per cent (compared with 17.5 per cent in compliant Britain).

Instead, more Japanese are paying old-fashioned taxes, and government receipts have been rising more quickly in recent years than any other economic indicators. (That could yet change in the wake of the recent stock market scandals and the deflation of the economic bubble.) The government may still be in the red, but the national debt is now slightly more manageable and, after years of fiscal restraint, civil servants are itching for a spending spree. So why not rebuild the universities?

There is a sense in which this is a soft option. Employing the much-needed extra technicians, maintenance and cleaning staff in chronic short supply would in the long run cost much more. A five-year splurge on new buildings (also necessary) is a more limited and also (if only just) an affordable commitment. And the benefits will be visible. Almost every overseas visitor to a Japanese university is dismayed at the abounding ostentatious evidence of neglect; some new departments, cosily supported by industrial contracts, are spick and span, but others seem not to have had the benefit of a lick of paint since the great earthquake of 1923. Renovation is long overdue, even if

able people needing extra help will continue to seek election to an extra chair for the sake of the extra pairs of hands that brings, and young researchers continue to do menial tasks that would be beneath their counterparts in the West.

The scale of the proposed renovation should also be appreciated. Monbusho now spends about \$666 million a year on building new universities and research institutes. It plans to top this up with \$111 million on rebuilding. Few research administrators in the West would not be carried away by such a windfall, even if some of the funds will come from Monbusho's research budget. And in any case, Japan, being Japan, will probably contrive to win creativity as well from its new investment. □

US kills sex survey

The NIH are afraid of both Congress and the Bush Administration when it comes to sex

A FEW weeks ago the US Department of Health and Human Services (HHS) vetoed a survey of sexual behaviour in teenagers that had won the approval of the National Institutes of Health. The questionnaire, the HHS Secretary said, undermined his efforts to promote abstinence. Now, the NIH themselves have backed off a survey of sexual practices among adults that had received high scores from a committee of scientific peers. The reason: NIH does not want to risk another confrontation with the Secretary, or the ire of conservative members of Congress who argue that what people do in bed is not the government's business.

In the midst of the AIDS epidemic, in which evidence points to the likelihood of an increase of virus spread among heterosexual men and women during this decade, a reluctance to learn about human sexuality in contemporary society and the circumstances that motivate people to engage in various forms of activity is just plain dangerous to the public health.

Two years ago, former British prime minister Margaret Thatcher, as narrow-minded on this subject as her American counterparts, thwarted similar studies. But British researchers succeeded despite the absence of government funding when a private foundation came to their aid. US researchers also deserve assistance from the private sector in order to gather valuable data in spite of the government. □



US budget: NSF wins, space science loses

■ Aerospace plane, big boosters cut to save Station

■ NSF, led by education, gets 11 per cent increase

Washington

RESEARCH lobbyists dread autumn in Washington. Almost anything can happen to the US science budget in the last weeks of September, when legislators rush to finish negotiations in time for the 1 October deadline. A year ago, the National Science Foundation (NSF) lost more than \$100 million when a plan to transfer the money from the Defense Department was vetoed with just hours to go.

Last week, in a House-Senate conference agreement, it was the National Aeronautics and Space Administration (NASA) that took the last-minute cuts, as Congress slashed plans for a new generation of large booster rockets and essentially abandoned the National Aerospace Plane (NASP) programme to save the Space Station.

While NASA science programmes were hit hard, basic research at NSF came out quite well. NSF received the largest total increase of any agency — 11.2 per cent. As expected, its popular science education programme won the largest increase — a 44 per cent rise to \$465 million in 1992. Research also did well, with an increase of 10.9 per cent to \$1,879 million. Surprisingly, funding for the Laser Interferometry Gravitational-wave Observatory was not deleted, so NSF will consider it a “live project” for startup in 1992, says NSF analyst Joel Whitter.

Another \$105 million of NSF funding still hangs in the balance. Earlier this month, the White House Office of Management and Budget (OMB) vetoed a plan to shift \$375 million from the Defense Department to NASA to cover military use of some NASA communications satellites. But it left open the question of who should pay for Navy support duties at the NSF Antarctic bases. NSF is assuming that the Navy will cover the \$105-million expense, but until the agency gets a firm word from OMB, last year's surprise veto of the same transfer remains a disquieting possibility.

The big question mark in both the NSF and NASA budgets has been the Space Station. After doing combat all year over whether to continue the \$37,000-million project in the face of technical problems that have cut its capabilities in half, Congress finally decided not only to do it, but to do it once and for all. The House-Senate conference granted the Station \$2,029 million — the entire presidential request. “It's finally out of the woods,” says John Logsdon, a professor of space and science policy at George Washington University in Washington, DC.

Congress's largesse on the Station came at a cost, however. Space science projects across the board will be cut, delayed or cancelled outright. The Advanced X-Ray Astrophysics Facility (AXAF) lost \$60 million and will be delayed a year. The twin space probes CRAFT-Cassini — one to intercept a comet, the other to visit

1992 congressional appropriations

(millions of dollars)

	1991	1992	
Selected programmes		<u>request</u>	<u>conference</u>
National Science Foundation			
Research	1,694	1,964	1,879
Education	322	390	465
Instruments & facilities	20	50	33
Antarctic	175	193	88*
NASA			
Research	6,023	7,198	6,414
& development			
Space flight	5,124	5,608	5,158
Space Station	1,900	2,029	2,029
NASP	95	72	5
NLS	24	175	33
AXAF	101	211	151
Life sciences	137	184	149
CRAF-CASSINI	143	338	211
EOS	187	336	296
Earth probes	52	48	68

*Pending the transfer of an additional \$105 for the Antarctic programme from the Department of Defense, whose budget is not expected to be finished until November.

Saturn — were both retained, although the programme as a whole was cut by \$117 million, which will delay Cassini by at least a year. LifeSat, a proposed reusable radiation-biology satellite, was terminated.

Among the programmes to escape the knife were the Earth Probes series of small remote-sensing satellites and the data-handling portion of the Earth Observing System (EOS). Congress earmarked \$15 million to start on a Climsat probe and \$5 million of additional instrumentation for the Tropical Rainfall Measuring Mission. It also added \$25 million of start-up funds

US RESEARCH POLICY

New direction for fusion

Washington

US FUSION scientists will have to rethink their programmes and goals and quickly come up with a new plan of action, now that funding has dried up for the Burning Plasma Experiment, planned as the next major step in fusion research (*Nature* 353, 287, 26 September 1991). The Fusion Energy Advisory Committee met for two days last week and came up with a set of

for the Consortium for an International Earth Sciences Information Network.

The big loser at NASA was the National Aerospace Plane (NASP), the planned hypersonic air/spacecraft that would be propelled from runway to orbit by hybrid jet-rocket engines. Congressional concern over the untested engine technology combined with budget pressures led legislators to conclude that research progress so far did not justify continuing with the programme. The House-Senate conference cut the programme to a burial-fee \$5 million. “They're basically saying ‘last guy out, turn off the lights,’” says John Pike, space analyst for the Federation of American Scientists.

Nevertheless, there may still be a glimmer of hope for the NASP. The programme is a collaboration between NASA and the Air Force, whose budget is still at least a month from completion. The National Space Council, led by Vice President Dan Quayle, has championed the project, and pressure from it could conceivably convince Congress to find enough money to save it in the defence budget.

Quayle has competition for his attentions, however. Earlier this year, his Space Council released a document outlining a National Launch System (NLS), which would combine the second-generation Shuttle C launcher with portions of the Strategic Defense Initiative (‘Star Wars’) Advanced Launch System programme. At an estimated cost of \$10,000 million by the end of the decade, the NLS was intended to provide a new family of heavy launch boosters that could carry payloads from 25 tons (the limit of current US launchers) all the way up to 300 tons. But short of the President's Mission to Mars initiative (which was again removed from the budget this year), no currently planned projects would require such a large booster. Although the President's January budget request had \$175 million for NLS, Congress last week left in only \$20 million to continue some engine development work.

Unlike the NASP, the NLS cuts are considered a temporary measure. With an ageing and inefficient fleet of shuttles, “there's a broad consensus that we need a new launch system,” Logsdon says. “The NLS will back.” **Christopher Anderson**

recommendations to the Department of Energy (DOE) on where to go from here.

The DOE, facing a projected decline in available research funds over the next five years, had asked the committee how it would deal with two possible scenarios: zero real growth in the coming five years, and five per cent annual growth. Committee chairman Robert Conn of the University of California, Los Angeles, said that

the first possibility would leave the United States with an "unhealthy" fusion research programme, but that the second is workable if the money is carefully spent.

The first thing the committee did, however, was to try to devise some way to save the burning plasma experiment, which is planned to succeed the Tokamak Fusion Test Reactor at Princeton University after work there is finished in two years. They concluded that the project would demand annual funding increases of 10 to 15 per cent and that stretching out its schedule would be impractical.

The major problem facing the US fusion community, Conn said, is that the removal of the burning plasma experiment leaves a gap between the finish of the tokamak experiment at Princeton and the beginning of the International Thermonuclear Experimental Reactor (ITER), during which time US scientists would not have enough to do to keep the research effort strong.

To fill the gap, the fusion advisory committee recommends that the DOE fund another major research effort, smaller in scope than the burning plasma experiment and inexpensive enough to fit into the confines of a fusion programme growing at five per cent a year. Ideally, such a programme would come on line three to five years before ITER and would offer operational experience for running ITER and also provide data that the later experiment is not designed to obtain.

Work on a proposal for the new project should be funded with part of the \$27 million already allocated in fiscal year 1992 for the burning plasma experiment, the committee recommends. After working with the US fusion community, the advisory committee should be able to offer one or more specific proposals by next August, in time to decide on funding for fiscal 1994, Conn said.

The loss of the burning plasma experiment will give ITER the major role in the US fusion programme and will also force a rethinking of how US researchers want to use ITER. Until now, US fusion scientists had generally expected the burning plasma experiment to provide much of the physical data necessary to plan a fusion reactor; in this case, ITER would be used more for testing technology than for doing basic science. Many European scientists, on the other hand, had hoped that ITER would concentrate more on physical experimentation. The Europeans, it now seems, will win this argument by default, as US researchers will have no place other than ITER to get their data.

The fusion advisory committee also recommended using some of the \$27 million to increase support for existing fusion programmes, many of which have not been operating at full capacity because of insufficient operating funds.

Robert Pool

Soviet space gloom

IN the next few weeks Soviet authorities are expected to cancel the Buran space shuttle project, which has seen only one launch, in 1988. Buran, which bears a striking resemblance to its US counterpart, is expected to be just one victim of wide-ranging cuts across the whole of the Soviet space programme, made necessary by the dire state of the country's economy. The centralized Soviet space programme is also threatened by the wish of individual republics — notably the Russian Federation and Kazakhstan, home to the main Soviet launch sites — to manage their own space programmes.

P.A.

Marine cats' eyes

THE Moray Firth in Scotland last week played host to field trials of a simple device designed to reduce the number of dolphins and porpoises killed in drift fishing nets — estimated by environmentalist groups to total 1.5 million a year. Researchers from Cambridge, Loughborough and Aberdeen universities hope that attaching sonar-reflective floats to fishing nets will increase their 'visibility' to feeding dolphins, and so prevent many from becoming entangled.

Margaret Klinowska, a Cambridge biologist, likens the reflective floats to the familiar 'cats' eyes' used to highlight road markings in the dark. The key to the success of the project, she says, is to make nets register on dolphins' sonar from a distance of 70-90 metres — the distance over which dolphins scan when searching for their fish prey. Conventional nets are detected by dolphins only once they are within 10 metres. Klinowska says that dolphins last week did seem to avoid a series of ropes spaced 2 metres apart each carrying reflective floats at 1-metre intervals, but it will take several weeks to analyse recordings of the dolphins' sonar pulses to discover whether the floats were detected.

P.A.

Japanese firms opt out

US PRESSURE on Japan to open up its satellite market to foreign (and in particular US) satellite manufacturers appears to have paid off. Last month, the huge domestic telecommunications company Nippon Telegraph and Telephone (NTT) announced that only US manufacturers submitted bids to supply a next-generation communication satellite to be launched for NTT in 1995.

Last year, during the US-Japan trade talks, Japan agreed under US pressure to open up its satellite market to foreign competition. Originally, the Japanese government had planned to build the NTT satellite as a national project led by the National Space Development Agency (NASDA), but this plan was dropped in the

face of US criticism. Japanese satellite manufacturers say they did not submit bids because they do not have adequate facilities for testing the satellite and they could not use NASDA facilities for fear of US complaints that their manufacture of satellites is subsidized by the Japanese government.

David Swinbanks

Animal supplier charged

IF the US Department of Agriculture (USDA) gets its way, the nation's largest supplier of animals for school dissection may soon feel the knife itself. In a complaint filed last week, USDA charged Carolina Biological Supply Company with embalming cats while they were still alive, improperly killing other animals and six other "wilful" violations of the Animal Welfare Act. USDA wants to fine the company and strip it of its licence. The charges stem from a 1990 investigation by undercover members of the animal-rights group People for the Ethical Treatment of Animals (PETA) who videotaped procedures at the company over seven days. In a statement released last week, Carolina Biological said that the cats were actually dead when they were embalmed, and were simply showing "involuntary, postmortem muscular movement". USDA has so far offered no evidence to the contrary, the company said, adding that it has taken steps to deal with USDA's other concerns.

C.A.

Leukaemia favours rich

CHILDREN from families of higher socioeconomic status are more likely to develop leukaemia than children from poorer families, according to a survey of almost 10,000 leukaemic children published last week by the UK Office of Population Censuses and Surveys. A University of Oxford team looked at cases over the whole of Britain diagnosed between 1966 and 1983, and correlated these with socioeconomic status at the level of county districts. Study leader Gerald Draper says the result confirms previous findings with smaller data sets, but the reasons for the link are still unclear. Draper speculates that the age at which children become exposed to viruses thought to trigger abnormal cell proliferation — likely to be later in children raised in more affluent families — may be an important factor.

Leukaemia epidemiology has been a controversial subject in Britain ever since clusters of childhood cases were identified around nuclear installations at Sellafield in Cumbria, Dounreay in Scotland, and Aldermaston and Burghfield in southern England. In the light of his results, Draper believes that the leukaemia cluster around the Aldermaston and Burghfield nuclear weapons research laboratories could be explained in part by the relative affluence of the surrounding area.

P.A.

In the wake of the coup

Moscow

THE failure of the attempted *coup d'etat* in the Soviet Union has brought increased freedom, but also an increased chance of chaos. The future of science and technology is still up in the air.

Russia's Silicon Valley

ZELENOGRAD, one of Moscow's satellite towns, is known as the 'Silicon Valley of the Soviet Union'. There much of the electronics work for the military is done, but the town was also a centre of rebels fighting the totalitarian system, and it has perhaps one of the brightest economic futures of any place in the country.

On 19 August, the day of the coup, as early as 9 a.m., L. Ivaniutin, general director of the scientific and production association Elma, spoke on the radio and called upon the personnel to boycott the junta's directive. Elma is the only Soviet producer of basic materials for electronics. For many years the enterprise had suffered at the hands of the All-Union Ministry, which fired or fettered the most intellectually gifted or active workers, but Elma will now come under the authority of the government of the Russian Republic. Several other Zelenograd enterprises are also getting out from under the authority of the military-industrial complex.

With the screen of secrecy torn off, it appears that the electronics industry is in mostly poor condition. Soviet computers are not competitive with foreign machines and cannot be sold even for the lowest prices, putting Soviet producers at the edge of bankruptcy.

Nonetheless, with its highly qualified specialists, Zelenograd is attracting foreign businessmen. For instance, the US company Cypress Semiconductor signed an agreement last year to manufacture computer chips designed by Soviet researchers and pay royalties for those designs. Cypress president T.J. Rogers, who visited Zelenograd last year, says that although the Soviet semiconductor manufacturing technology is six or seven years behind that of the West, their design work is excellent. "I saw a dozen ideas that would have triggered start-ups in Silicon Valley that were just sitting on someone's desk," he says.

Rogers says he was approached by Soviet chip designers who pleaded with him to manufacture their circuits, which are too advanced to be made with Soviet equipment. He has high hopes for future cooperation, but "with all the turmoil going on, we haven't really gotten too far."

Unfortunately, the Soviets have little more than design skills to offer for joint ventures with the West. They can provide the land for production facilities, and perhaps some obsolete equipment for low-

cost assembling operations. But although difficulties lie ahead, there is a great hope that the city of Zelenograd, which stood up to the *coup d'etat*, will have the same persistence in solving economical problems and raising the scientific and technical level of production.

Who will own Mir?

THERE are widespread rumours that one of the best Soviet space vehicles, the orbital space probe Mir, is for sale. Newspapers have even mentioned the approximate asking price: \$600–800 million.

Yu. Semenov of the scientific and production association 'Energia', which specializes in the development, production and operation of space equipment, denies these rumours. He does admit, however, that even the once-inviolable Soviet space programme has not been left untouched by the chaos wracking the country. So what will happen to Mir?

Sharp cuts in state allocations have forced the space programme to seek new sources of revenue. Semenov believes that the only way out of the present situation is



to create joint-stock companies with foreign companies and to transform Mir into an international scientific laboratory. "We have got several offers already, some of them quite serious," he says, so Mir is at present in no danger of being grounded. "We shall certainly fulfil all our obligations concerning the space flights of Austrian, German, and French astronauts. Austrian astronauts will start off as soon as October 2."

Energia is planning two more modules to dock with Mir, one for optical studies and one to do ecological work. By 1994, after a rearrangement of the modules, the association hopes to change the basic unit and to start the construction of a second-generation Mir probe, which should be finished by 2000, assuming that money is found for the project.

Financial problems are not the only ones facing Mir — even the ownership of

the station is in doubt. Decades of so-called 'public ownership' in the Soviet Union have created a situation in which nobody knows who the real owner is of this or that property, and successful privatization of the space industry will be of no less importance than privatization on land.

Helene Knorre

LONDON ZOO

Management under fire

London

THE embattled management of London Zoo came under attack last week from many of the fellows of the Zoological Society of London, who argued that the present team must be replaced by a crisis management committee if the closure of the zoo's Regent's Park site is to be prevented.

At the end of a turbulent annual general meeting, a motion of no confidence in the zoo's management was dismissed by society president Avrion Mitchison on a procedural point. But supporters of that motion plan to request another meeting of the society before the end of the year, to ask for changes in the zoo's management.

Colin Tudge, a writer on zoology and conservation, says the London Zoo has been travelling down the wrong path for the past decade: while other leading zoos have streamlined their collections, basing public exhibits around their work in captive breeding and conservation, London has essentially remained a "nineteenth century menagerie". The present efforts by the zoo's management to attract more than £12 million of investment to convert the Regent's Park site into a centre housing fewer animals and stressing the Zoological Society's work in conservation could be a step in the right direction, says Tudge, but it is one that should have been taken several years ago.

David Jones, who heads the team that runs the zoo's two sites for the society, says replacing the management now would jeopardize negotiations with investors aimed at securing the zoo's financial future.

But Jones's critics say it is the continued presence of the present management that has stymied attempts to attract funds from the government and the private sector. Trevor Poole, society fellow and deputy director of the Universities Federation for Animal Welfare, wants the zoo's management handed over to a small committee of experienced directors from other British zoos, many of which are financially healthy.

The governing council of the Zoological Society has so far shown no sign of relinquishing its support for the present management. But if no promises of major private investment are made in the next few months, that loyalty may be tested. "I wouldn't rule out any options," says Peter Holwell, principal of the University of London and the society's new treasurer.

Peter Aldhous

Europe weighs a carbon tax

London

THE member states of the European Communities (EC) must decide whether they are prepared to take real — and possibly costly — action to meet their stated commitment to stabilize the EC's emissions of carbon dioxide at 1990 levels by 2000. Last week, to that end, the European Commission proposed a formal draft directive to impose an EC-wide tax that would add \$10 to the price of a barrel of oil by the turn of the century.

The proposed tax would penalize both the burning of carbon and the use of energy in general. The \$10-per-barrel oil surcharge would be split equally between a tax on the fuel's energy content and a separate levy on the amount of carbon dioxide produced when the oil is burned. Carbon-rich fuels, such as coal, would be taxed more heavily, but even nuclear-generated electricity would be subject to the energy levy; the only exemption would be for renewable energy sources. The Commission's intention is to introduce the tax gradually, starting with a \$3 tax on a barrel of oil in 1993 and increasing by \$1 a year over the rest of the decade.

In Brussels, officials under EC environment commissioner Carlo Ripa di Meana have been developing the proposal for many months, and the version given the full Commission's backing last week contains a number of 'sweeteners' designed to lessen opposition from the member states. Many EC member governments would have objected to the idea of a new tax swelling the Commission's coffers, but the new proposal requires that the carbon/energy tax be fiscally neutral: the tax would be collected by the member states' governments themselves, and the extra income should be offset by reductions in other national taxes.

Energy-intensive industries relying heavily on international trade — such as chemicals, iron and steel, and paper manufacturing — may also be protected from the tax if the EC's international competitors do not take similar measures. According to one Brussels source, these industries could be exempted from the tax altogether if the United States refuses to adopt a target to stabilize its carbon dioxide emissions.

But even with these concessions to

CLARIFICATION

SHIRLEY McGreal, chair of the International Primate Protection League, asks that it be made clear that she did not (as reported in *Nature* 353, 199; 19 September 1991) ask recipients of her letter "if they should be doing business with" Matthew Block, but that she merely asked them to "peruse" a notice issued by the Centers for Disease Control to Worldwide Primates Inc. listing 46 instances of non-compliance with quarantine regulations.

ensure international competitiveness, the tax is expected to have some damaging economic effects, including an increase in the EC's annual rate of inflation by as much as 0.5 per cent. This may deter some EC member governments from supporting the measure. British environment minister David Trippier said last week that Britain is not yet ready to support the introduction of a carbon tax, and he suggested that two-thirds of EC member states back the British position. Some governments, such as the French, are violently opposed to any EC interference in national taxation policy, he added. But with the Netherlands, a strong supporter of the carbon tax proposal, holding the EC presidency, the draft directive is certain to be high on the agenda when the member states' environment and energy ministers meet over the next few months.

Officials in the Commission's environment directorate-general reject Trippier's assessment that the proposal

lacks support, and argue that the tax is necessary if the EC's commitment to stabilize its carbon dioxide emissions by 2000 is to be met. According to the Commission's economic models, an \$18 surcharge on a barrel of oil would be needed to meet this target, although Ripa di Meana's staff believe that the \$10 levy can do the job if combined with other measures, such as stricter standards for energy efficiency.

Whether or not the EC member states accept the new tax, the EC must still pursue its efforts to convince the US government to adopt a target to stabilize its carbon dioxide emissions. (Stabilizing emissions is itself only a small first step towards halting the build-up of carbon dioxide in the atmosphere, and so preventing the expected global warming). The Bush Administration has repeatedly rejected the notion of emissions targets, arguing that the scientific evidence for carbon-dioxide-driven global warming is still too tentative to serve as a basis for firm commitments to specific reductions in greenhouse gases.

Peter Aldhous

EUROPEAN SCIENCE POLICY

Research given priority in new French budget

London

SCIENCE fares well in the French national budget for 1992, announced by the Council of Ministers late last month. With total public expenditure due to increase by only 3.1 per cent (near the predicted rate of inflation over the coming year), the proposed FF51,000 million budget for civil research and development is 7 per cent more than in 1991.

Officials at the Ministry of Research and Technology, which is responsible for just over half of French government spending on civil science, say the French government is making research, education and the environment its main priorities, and they expect the budget to be approved by the parliament with no major changes. The new budget marks a return to steady growth in French science spending, after a hiccup earlier this year when some FF1,000 million of the planned 1991 research budget was trimmed by the government (*Nature* 350, 181; 21 March 1991). Those cuts, which affected the entire spectrum of public spending, were made necessary by the recession and expenditure on the Gulf War, and are not expected to be repeated next year.

Academic researchers should be happy with the proposed 1992 budget, which gives

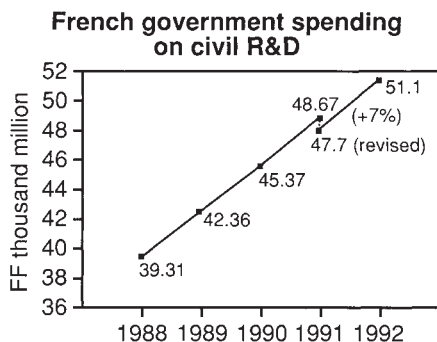
FF11,852 million to the Centre National de la Recherche Scientifique, the principal government agency supporting basic scientific research, 8.1 per cent more than its allocation for 1991. But the real winners are French industrial scientists, who will receive FF6,100 million, 17.5 per cent more than in 1991. In addition, tax credits given

to companies carrying out research — not included in the budget figure — are set to rise by almost 10 per cent, to a total of FF4,500 million. Already, the French government's generous support for industrial research has already caused some raised eyebrows in other European

Communities states, which fear that French companies may gain an unfair competitive advantage (*Nature* 353, 98; 12 September 1991).

There is one area of stagnation in the new research budget, however. The French atomic research agency — traditionally strong, given the country's 70 per cent reliance on nuclear-generated electricity — faces a budget that will grow by only 0.3 per cent over its 1991 allocation, to FF6,300 million. After inflation is taken into account, France's nuclear researchers will find themselves with less money to spend.

Peter Aldhous



Japan reaches out

Tokyo

In its annual policy statement, the Japanese Science and Technology Agency (STA) recommends globalizing Japan's scientific and technological research to counteract a perceived movement toward protectionism in Western countries, in particular the United States. The white paper closely echoes the policy of 'technoglobalism' announced by the Ministry of International Trade and Industry (MITI) last year, and indicates that Japan is serious about internationalizing its research activities.

The document, submitted to the Cabinet last Friday, says the country should follow this course "even if there should occur protectionist attitudes in view of the steady advance of Japan", because globalization is a "plus sum game" from which "all can benefit in the long run".

Such internationalization has already begun, the white paper shows. Since the mid-1980s, many Japanese high-technology companies have established overseas research and development centres. An STA official says there are now 276 such facilities, mainly in the United States and Western Europe. Most of the research institutes are fairly small, with fewer than 50 researchers, but the agency found that many of them plan to expand to 50 to 100 researchers by the mid-1990s.

The electronics company NEC, for instance, recently established a small basic research institute in Princeton, New Jersey, in the United States. NEC officials say they received a flood of applications from high-quality researchers, many of them from leading US industrial research centres. Now NEC is planning to set up another research centre in Europe, in either Germany or the United Kingdom, to tap talent there.

Japanese companies are also beginning to recruit non-Japanese scientists to

their institutes in Japan. The total number is still small — 751, according to the white paper — but it has tripled in the past three years. About half come from Western Europe and the United States, and the rest from Asia.

Furthermore, the number of postdoctoral fellowships offered to foreign (mostly Western) scientists by MITI, STA and the ministry of education has risen from a few dozen several years ago to 369 this fiscal year. And the agencies plan to expand the programmes.

Despite this growing influx of Western scientists, the gap in exchange of researchers with the West on both a short- and long-term basis continues to increase because many more Japanese scientists and engineers are going to Western countries. On the other hand, 20 per cent of graduate students in Japanese universities in 1990 were from overseas, up from 10 per cent in 1985, but most are from Asian countries.

Some shortcomings do exist in Japan's globalization initiative, the white paper finds. For instance, it says that Japan's exchange of information with the United States and Europe through computer networks — such as the Scientific and Technical Information Network, which links the Chemical Abstracts Service of the United States, the Japan Information Centre of Science and Technology, and Fachinformationszentrum-Karlsruhe in Germany — is much more limited than the exchange between Europe and the United States.

The white paper also says that discrepancies in intellectual property rights among countries deter globalization of research, and it calls for making patent systems consistent. MITI has recently rewritten Japan's laws to allow foreign companies access to patents arising out of Japanese government projects they participate in.

David Swinbanks

But are manufacturers still holding out?

Washington

WHEN the customer has been a US company and the product was state-of-the-art technology, Japanese manufacturers have repeatedly delayed sales, charged much higher prices or refused to sell the products at all, an 18-month investigation* by the congressional General Accounting Office (GAO) concluded last week. This finding is in marked contrast to the open attitude that Japanese government and industry are trying to encourage (see above).

About half of the 59 companies GAO interviewed (under promise of anonymity) gave examples in which Japanese refused to sell them electronic or semi-

conductor-fabrication technology or delayed their orders by more than six months. One company said that its attempts to buy a piece of etching equipment were rebuffed for a year while the Japanese company said the product was still being test-marketed in Japan and that US service and English-language manuals were not yet available. Most of the US companies thought that the Japanese response was understandable, however, and argued for greater US technology independence rather than sanctions against the manufacturers.

C.A.

* US Business Access to Certain Foreign State-of-the-Art Technology, GAO/NSIAD-91-278, September 1991.

Canada advances KAON

Washington

LATE last month, the Canadian government announced that it is committing \$236 million for construction of KAON, a major upgrade of the existing TRIUMF cyclotron at the University of British Columbia. Prime Minister Brian Mulroney's administration describes the support as new money — separate from the \$1,500 million already budgeted for science and technology.

The proposed KAON accelerator would use TRIUMF (the Tri-University Meson Facility) to accelerate proton beams to 520 MeV and then inject them into a series of new accumulator and accelerator rings which will boost the proton beams to 30 GeV. This beam will then strike a target, producing kaons, antiprotons and neutrons. Kaons are valuable because their mean lifetimes are long enough to allow researchers to assemble enough of them to provide high statistical accuracy. Hypothesis-testing experiments that would take several years on existing facilities will take only hours on KAON.

The KAON decision has disturbed many Canadians because of the political circumstances surrounding it. British Columbia's incumbent conservative party is mired in ministerial scandals involving corruption, breach-of-trust and fraud. The announcement of the KAON award from Mulroney's federal conservative government was made hours before a provincial announcement of an election for 17 October.

TRIUMF's director Erich Vogt denies politics played a role in the timing of the announcement. He received word several weeks ago to expect good news, he said.

Others are more sceptical, given that science advisory committees have recommended against KAON unless there is a commitment of long-range support. "It does draw into question the role and function of the advisory bodies, given the unanimous recommendations not to proceed with KAON," said Janet Halliwell, chairman of the Science Council of Canada.

The federal government's decision to commit itself to KAON comes at a time when support for the project is still uncertain. When added to a previous \$236 million commitment from British Columbia, the federal commitment will promise about two-thirds of KAON's projected \$709 million capital costs, but there are no guarantees about the final one-third of capital costs or the operating expenses.

The charge that politics influenced the KAON decision rings somewhat hollow, given that the TRIUMF-KAON project has never been considered merely as science. Most politicians support it as part of an effort to redistribute institutions and facilities — and the employment opportunities they provide — across Canada.

Catherine McMullen

Three blacks on research council

Cape Town

THE South African government has added black representatives to the Scientific Advisory Council (SAC), which advises the cabinet on how to allocate science funding among the various science councils and national facilities. Three of the 13 members of the present council, which met for the first time on 27 August, are black academics.

The SAC does remain, however, safely under the control of the old guard, with the appointment of Chris Garbers to a three-year term as its head. Garbers served for ten years as president of the Council for Scientific and Industrial Research until he retired about 18 months ago.

The three new black members are A.C. Nkabinde, rector of the University of Zululand; Bob Seretlo, dean of the science faculty at the University of Fort Hare; and, perhaps most significantly, Mamphela Ramphele, a newly appointed vice-principal of the University of Cape Town. Ramphele, a medical doctor, was a former close associate of Steve Biko, the black leader who died while in security police detention in 1977. A relative 'radical', Ramphele is unlikely to always agree with the government's official policies.

Opening the new council, the Minister of National Education, Louis Pienaar, offered some hope that things may improve for the South African science budget, which has been declining in real terms for several years. Warning against the perils of cut-

SUPERCONDUCTORS

Fullerene false alarm

Tokyo

A GROUP of Japanese researchers has retracted a claim to have developed a superconducting fullerene with a record-breaking critical temperature of 57 K. The result, which was reported around the world (*Nature* 352, 749; 29 August 1991) and sent researchers scurrying to repeat it, was apparently an artefact.

After announcing the claim, the group at the National Research Institute for Metals was flooded with enquiries from researchers unable to duplicate the results. The team, led by Hisashi Sekine, then tried to repeat the experiment, but without success. Eventually they realized that in the original experiment one of the team had put the fullerene samples in the wrong coil of a superconducting quantum interference detector (SQUID) used to measure magnetic fields.

A paper on the research due to be published next month in the *Japanese Journal of Applied Physics* has been withdrawn, and in a press release the institute apologizes for the trouble caused to fullerene research.

David Swinbanks

ting funds for science, he said, "If there is one area that should not be hurt through hasty actions, it is our science system." The scientific community is taking these sentiments with a grain of salt, however, since shortly after Pienaar was appointed as minister, South Africa experienced worse-than-usual cuts in budgetary allocations to the research councils (*Nature* 350, 549; 18 April 1991); those cuts were the first ever in nominal terms, as opposed to real terms.

The SAC's most important function is to advise the cabinet on the distribution of the science 'vote', or budget, among the five statutory councils and the three national facilities — the National Accelerator Centre, the South Africa Astronomical Observatory and the Hartebeesthoek Radio Astronomy Observatory. (The national facilities are part of one of the councils, the

INDIA

Death pills from pesticide

New Delhi

A LOW-PRICED, deadly pesticide has become the suicide agent of choice in India, alarming doctors and social workers, who say it has made suicide too easy. The pesticide is sold in the form of 3-gram tablets packed in a plastic phial about the size of a pen. Over the last five years, more Indians have taken their lives by consuming this death pill than died in the gas leak from the Union Carbide pesticide plant in Bhopal in 1984.

Because it is efficient, easy to apply and very cheap, aluminium phosphide (ALP) has been the pesticide of choice for fumigating stored grains in India. Although it is meant for use in government silos exclusively by government officials, ALP has entered the retail market and emerged as the most common suicide agent. It has supplanted all other types of poisons used for suicides in most Indian states, and central forensic science laboratory sources say it is also increasingly being implicated in homicides.

The toxic effects of ALP are due to the deadly phosphine gas liberated when it comes in contact with moisture in stored grains. Two tablets of ALP can protect one ton of wheat from pests. When consumed by humans, it reacts with hydrochloric acid or water in the stomach, releasing the poison gas. A single tablet gives off one gram of phosphine, ten times the lethal dose. Death follows in two hours and ALP has no antidote — attributes that have raised its popularity as a suicide agent even among illiterate villagers.

Doctors and social workers have demanded tighter control on its use and have even called for a ban on production of ALP. The government admitted in the

Foundation for Research Development, but are run on separately earmarked funds.) The SAC's recommendations are not made public, so whether budgetary allocations reflect them or overriding decisions by the cabinet is always a matter of conjecture. The proportion of the vote allocated to each council remained roughly constant from 1985 until this year, when cuts varied slightly among the councils.

Pienaar's speech specifically asked the new council to investigate the views of "new and important role players on the political scene" on science policy, and advise the government accordingly. With changed priorities in South Africa, the advisory council may decide to allocate resources differently. The science vote will be increased next year to provide for a new statutory council, the Agricultural Research Council, to be established on 1 April, so the SAC could use the opportunity to reassess the country's scientific priorities.

Michael Cherry

parliament last week that deaths due to all pesticide poisoning increased from 192 in 1988-89 to 664 in 1989-90, but ruled out a complete ban on ALP as it might offset the gains of green revolution. Unofficial figures of pesticide-related deaths are much higher.

Reports of ALP poisoning — unknown before 1980 — have come in from hospitals from several states of India. A single hospital in Rothak in the state of Haryana received 418 cases in a seven-year period, of whom 286 were attempted suicides. According to doctors of the hospital, incidence of ALP poisoning in that state increased from 0.2 per thousand hospital admissions in 1982 to 3 per thousand in 1986 and 5 per thousand in 1987. In fact, every suicide victim brought to Indian hospitals is assumed to have consumed ALP. And no autopsy of such cases is done without a gas mask, after a reported incident where an autopsy surgeon had to be given first aid after being hit by a jet of phosphine from an excised stomach.

Three companies manufacture ALP and the entire production is purchased by the government. Under the official guidelines, ALP should be used only by government grain storage facilities, agricultural departments and authorized pest control operators and cannot be sold in retail outlets for individual use. But these guidelines were not sufficient to prevent the entry of the death pills into the retail market through pilferage or deliberate leaks. Admitting that ALP is sold through unauthorized channels, the government last week announced it would be taking stricter measures to plug the leaks and also asks the manufacturers to pack the poison in pilfer-proof packages. **K.S. Jayaraman**

Problems of British science

SIR — One of the major points not addressed in your Manifesto for British Science (*Nature* 353, 105; 1991) is that of an adequate career structure for British university academics which, of course, goes wider than science and technology. The small proportion of senior academic (that is, professorial) posts at British universities means that many distinguished academics cannot rise above the normal career lecturer grade. In contrast, in the North American university system, academics are periodically assessed for promotion on merit on an *ad personam* basis, through the ranks of assistant-, associate- and full-professor, with the latter not necessarily being tied to the headship of a department, as is mostly the case in the United Kingdom. Indeed, the North American system of democratically elected, rotating chairmanships of a department, usually chosen from the ranks of associate- and full-professors, is barely in its infancy in Britain. For better or worse, most UK university departments are run by professors (in the British terminology) elected until the retiring age, whose activities necessarily become dominated by administration.

I believe that the lack of realistic promotion prospects is one of the main reasons why young, promising, suitably qualified British academics seek their fortunes abroad. A high proportion of postdoctoral workers from my own research group have done so with great success over the past 30 years, and would not contemplate returning to the United Kingdom under present conditions. All British university departments are familiar with this situation. The relative lack of promotion prospects, rather than money alone, is one of the main causes of dissatisfaction among British academics, and it is frequently referred to when one visits expatriates working abroad.

In recent years there has been a move — for example in some Earth science departments, after their recent national reorganization — to head-hunt distinguished senior British academics who have deservedly made the grade abroad, by offering them incentives far superior to what they could have expected if they had remained in the United Kingdom in the first place. In my view, it would be more effective if a higher proportion of promising academics had the opportunity of fulfilling their talent and potential in the United Kingdom right from the beginning and throughout their careers.

Some aspects of the British career structure for university academics have clearly outlived their usefulness. It could be that the main reason why this impor-

tant problem is rarely addressed by the relevant committees, organizations and institutions within the United Kingdom is that the people in the best position to initiate change — most of whom are well acquainted with the North American system — also have the highest incentive to maintain the status quo.

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SIR — At this large Medical Research Council (MRC) unit where I am director of studies, we interview many prospective graduate students and ask them to talk about their honours projects. The standard is impressive and derives from the students having learnt a wide range of skills in completing a 3–6 month laboratory project under an active research worker.

If departments are to take on many more honours students, as they are being encouraged to do, it will not be possible for them all to undertake experimental projects because of a lack of supervisors, laboratory space and equipment. Instead, students will have to produce literature surveys for their honours projects and will thus end up being far less well trained in experimentation than they are now. The net result will be that graduates will leave the universities with little real laboratory experience and, perhaps, diminished enthusiasm for their subject. For the few who intend to do PhDs, this ignorance will only slow their future progress, but, for the majority going into industry, matters are far more serious: companies will have to provide the facilities to teach their new employees the laboratory skills that they have hitherto taken for granted.

Unless extra funds are provided for university staff and laboratories very soon, more honours students in science will mean worse students, irrespective of their ability and of whether they do three- or four- year degrees.

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SIR — Your recent Manifesto for British Science was certainly thought-provoking. However, it was grossly inaccurate in one important element, repeated in your leading article on 19 September (353, 195; 1991), involving the relationship between the Ministry of Agriculture, Fisheries and Food and the Agricultural and Food Research Council (AFRC). The ministry's contribution to the AFRC's budget is not "virtually zero"

and we certainly did not "simply walk away" from our contract with the council. We have a close working relationship, as befits the two largest funders and practitioners of agricultural and food research in the country. And the ministry does continue to fund the council. Last year our commissions with the council came to £45 million, 30 per cent of its budget.

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SIR — There is discontent with the present method of allocating British research council project grants that seems to run deeper than the grumbling that one might expect from people who think they deserved a grant.

The universities and similar institutions are now engaged in a deadly game of Strip Jack Naked, based in part on research ratings which will determine their access to other funds. Ratings depend in turn on success in obtaining research council grants. The system is intended to approximate to a free market.

But in what free market is a trader obliged to reveal all his sources and ideas to a panel of rival traders? Research applications are placed before referees and committees comprised of representatives of the very institutions that will benefit from the failure of the application.

At the very least, panel members and referees can and do take advantage of reading grant applications for improvement of or downright incorporation into their own applications; as the 'unknowing' of something one has read is impossible, their own publications and research clearly benefit from the sight of rejected applications.

Anonymity and confidentiality meanwhile ensure that referees and panels can make self-interested or more often plainly ignorant or misguided criticisms of applications that they would never dare to put their name to on the open page, and to which the applicants have little access or right of reply.

This set-up is certainly stifling good and original research, and is not improving the atmosphere. It is hard to understand why the relevant authorities contemplate it with such complacency. The importance of 'getting it right' will increase as central research money is increasingly removed from 'dual funding' and transferred to the research councils.

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A future for British science

Mark Richmond

The speech by Sir Mark Richmond made at the recent meeting organized by *Nature* has been much quoted. Here we present the text.

TRADITIONALLY, research at British universities and polytechnics has been sustained by what we have come to know as the dual support system. Direct support for research projects is provided by the research councils, while higher education institutions (HEIs) have provided general support from their recurrent and capital budgets. Over the years since the Second World War, we have done well out of these arrangements. But now I believe the system is under threat. This is a matter of crucial importance to the future well-being of science in Britain.

What follows is my personal analysis of the present strains on the system, and my opinions of what must be done to safeguard our system for supporting research in Britain. My views and opinions are mine alone; they are not the policy of the Science and Engineering Research Council (SERC), of which I am the chairman.

It is important, at the outset, to be clear what the funding of science through the research councils and our HEIs seeks to achieve. I believe the aim is primarily twofold: first, the provision of highly and appropriately trained manpower; and second, the nurturing of a strong research base across a wide range of disciplines. For the second purpose, the concept of critical mass is important: support must not be spread so thinly that it is ineffective.

Pressing need

The British science base is funded very substantially from the public purse, but there are other substantial claims on the same funds, many of which are regarded politically as more pressing than the support of science. So the science carried out with public money must be, and must be seen to be, of real need and importance. That must be measured, I believe, by the future well-being of the country's science base and of the people who will enrich it. It must not be valued by short-term advantage.

In particular, the support of science from the public purse should not seek immediate commercial gain. What we need is a strong central activity in high-quality fundamental science carried out by gifted scientists. Then, the exploitation of scientific discoveries by industry and commerce should be a process akin to osmosis rather than to a rigid linear coupling of 'cash in to cash out' and so to

profit.

One example of how well this system can operate is in the interaction between the British pharmaceutical industry and the science base: although some universities and polytechnics are engaged in focused contract work seeking defined pharmaceutical products and short-term gain, most of the interaction is between strong research groups in HEIs and complementary groups in the industry, acting more at arm's length. Few individual products arise immediately and directly, but many of the areas of profitable in-house investigation by the companies do originate at HEIs. The interaction across the industry/science-base interface is intense and effective.

In setting objectives for the dual support system, it is proper to put the provision of trained manpower before the support of experimental work. The two are strongly linked, but without a cohort of gifted researchers in the years ahead, can the continuation of the science base be possible? And if that fails, can industry-based research survive?

A strong research base sustained by gifted and motivated people also brings great prestige to a country and is a huge asset to its cultural life. I believe these are aspects of the support of science greatly underestimated by our political masters. The sharp difference between attitudes in Britain and those in the rest of Europe is already conspicuous. The arrival of 1993 can only accentuate the difference.

Since the Second World War, the twin objectives of trained scientific manpower and an excellent research base have been sustained by the dual-support system. The arrangement is that the government funds the universities to provide well-found laboratories into which the research councils inject grants to individual research workers. The system has worked extremely well. The output has been a stream of gifted young researchers and much excellent science. The system is much admired overseas and frequently copied.

The research councils differ greatly in the extent to which they contribute to the dual-support system. Some primarily support their own research institutes outside the HEIs; others make most of their awards to individual research groups in higher education. Of the councils, SERC deploys by far the greatest resources within the dual support system. This council, together with the Universities

Funding Council (UFC), is inevitably the main motor of research in our higher education system.

Hitherto, only the university sector of higher education has been funded to play a part in the dual-support system. This arrangement has been much criticized by the polytechnics, which regard it as providing preferential support for the universities. Part of the impetus for the current drive by the polytechnics for university status is their desire to see dual support extended beyond the present universities. Whether these aspirations will be fulfilled remains to be seen. But it is crucial that the system now in place in the university sector should not be further weakened.

Starved research

Despite its past achievements, the dual-support system is now being seriously undermined to such a degree that the future of good scientific research in our HEIs, and the future provision of excellently trained researchers, has become problematical. Increasingly, research groups and research institutes outside the university sector are seen to be the pre-eminent locations for high-class research, and potentially excellent researchers in our universities are emasculated by the increasing demands of undergraduate teaching and of administration. Moreover, the failure of the government fully to reflect in its allocations of funds the march of real-cost inflation in research means that university research is gradually being starved out of existence.

The attrition of the universities' part in the dual-support system has been going on for longer than on the research council side. For much of the 1980s, indeed, government funding of research through the councils was relatively buoyant. But the research councils are now also under strain, which is further eroding the dual support system.

Quantitative evidence to support these contentions is not easily marshalled, largely because research support by our universities was, until recently, totally undefined. (The sums involved are still not fully clear.) It would also be a major task to define the real cost of carrying out research and to analyse its change with time. But so daunting has become the task of protecting the research base in universities that some institutions are now spending on average at least 60p

from their own funds for every £1 awarded by the research councils, compared with the expectation that institutions would contribute only around 35p for £1 of council support. Yet even this subsidy does not match the need, and has been made possible only at the expense of support for teaching.

There is another indirect measure of the erosion of dual support. The research funds sought by the universities from the research councils grew rapidly between 1980 and 1988, while the number of teaching staff at universities was approximately constant. This is usually interpreted as a pressure to shift the balance between the two components of the dual-support system — away from the universities and towards the research councils. But the number of research grants awarded by the research councils during the same period shows that the hopes of the universities are largely unrealized.

Other demands

Inadequate funding on the university side has placed greater demands on the research councils in other ways. Over the past few years, requests for items that would previously have been provided by universities (for example, technicians, small items of equipment) have increased steadily. SERC has even altered its policy to help make good shortfalls in the provision of well-found laboratories.

Another sign of strain is the growing practice of funding equipment jointly, by universities and the research councils, often by means of centres serving several institutions. Although such equipment centres may often be justifiable on scientific grounds, and may foster interdisciplinarity as well, the trend reflects a shortage of resources in both components of dual support.

The provision of high-resolution mass spectrometry machines is a case in point. No chemistry department aiming at world-class research should be without one. Yet SERC now helps to fund one national centre (at Swansea) and five or six dedicated machines in individual departments.

So may not the continued survival of the dual-support system be impossible under reasonable expectations of science funding in the future? Should the objective of focusing scientific research within British higher education by means of dual support instead be abandoned? Some now openly ask these questions, proffering further development of institutes and units directly financed by the research councils as an alternative.

I believe that to move in that direction would be disastrous. High-class scientific research of the type needed to maintain a robust research base for the present and the future must be located in our HEIs.

Only then will there be the necessary close coupling between research and the flow of young men and women capable of high-class research. Indeed, I believe that a robust and well resourced dual support system is a *sine qua non* for the provision of opportunity to our young researchers; and impaired opportunity is one of the most baleful influences on scientific research in our universities now.

It is against this background, then, that urgent action is needed if the research base is not to be irreversibly damaged. At the heart of the problem, I believe, is the question of morale. This is a concept not much liked by scientists because it is difficult to make quantitative; and it tends to be dismissed by politicians because its implications are unpalatable. A fall in morale hints at a failure of policies; and that may be reflected in votes in due season. Nevertheless, I believe the demoralization of researchers in our universities is real enough, and I am impressed, as I visit universities and polytechnics, by how demotivated many research staff seem to be. The situation requires urgent attention. At the very least, poor morale leads to poor value for public money. At worst, it induces practitioners to leave the field or to emigrate, and it discourages the gifted young from a life of research.

All is not lost, of course, nor is everything doom and gloom. British workers are capable of research of the highest class by international standards; many are still thus engaged. Not all our best workers have joined the brain drain — although many of high quality have. But urgent action is needed if the decline is not to become terminal. Excellent research is less widespread than formerly, and our international standing is being eroded. And this is happening when those with whom we naturally compare ourselves are developing their research strengths rapidly and effectively.

Central to the question of morale are two more quantifiable issues: opportunity and remuneration. For gifted young people to be attracted to a career in science, they must have the facilities and environment within which they can exercise their talents. And they must be paid at levels that do not require total abandonment of rationality. Unless these conditions are met, the science base will not be developed.

Both the provision of opportunity and the improvement of remuneration will require extra resources, but the prior need is that everything possible be done to maximize the strength of the dual support system with the resources now available.

The financial component of the dual support system channelled to universities through the Universities Funding Council needs to be defined and quantified; in-

stead of a single sum for teaching and research, two sums, one for each, need to be defined. Only then can it begin to be known how much is being deployed by the universities within the dual support system — and how effectively it is being used.

Some progress has been made. Since 1986–87, allocations to individual universities against research-based and teaching-based criteria have been identified separately, but even now a university is not compelled to spend research money so designated for research. Yet failure to do so is likely to weaken the dual-support system.

It seems probable, at least for universities with large research-grant incomes, that some unknown proportion of the funds allocated against teaching-based criteria have in the past been used to support research. At institutions with relatively low research incomes, practices are even less certain. And at all institutions, there is no assurance that research money is exclusively focused on work of the quality needed to sustain a research base of the necessary excellence. In short, the effective coupling of UFC research funds with research of excellent quality in universities could undoubtedly be improved. This issue needs to be pursued energetically if funds made available for research are not to be wasted.

Complications

A further complication is that the research-based criteria used by UFC to determine a university's research allocation do not all relate to research as such. Much of it (on the average, 39 per cent of the total research-based allocation in 1990–91) relates directly to undergraduate student numbers. This means that an increase of funded undergraduate places at a university attracts extra research money even though undergraduates do virtually no research. This arrangement has meant that a number of institutions have gained extra research money in the current year (1991–92) without any evidence that the quality of their research has improved. Inevitably, as some institutions have gained funds for research in this way, others — particularly universities doing excellent research but electing not to increase undergraduate numbers — have lost research money.

This cannot have been what was intended, but the effect has been to weaken the dual-support system. Even if undergraduate members are taken as a proxy for academic staff numbers, the logic is unsatisfactory. Not all universities have recruited extra academic staff to match increased numbers of undergraduates. Rather, many universities are letting staff/student ratios deteriorate;

and this puts pressure on the time an academic staff member has for research.

There is reason to believe that the inconsistencies of the funding arrangements for university research — for the university side of the dual-support system — have now been recognized by the Government and UFC; it seems probable that appropriate corrections will be made in future. To state the matter unambiguously: there is an immediate need, for the health of the dual-support system, totally to uncouple allocations for research from undergraduate numbers.

The well-being of the dual-support system also requires increased selectivity and concentration of research support — funding only the best research within the means available (selectivity) and the deployment of resources only on groups large enough to allow them, in their fields of research, effectively to train people and pursue research (concentration).

Selectivity has not yet gone far enough. Indeed, as with the inappropriate coupling of research funding to undergraduate numbers, some recent policy innovations have had the opposite effect. A further difficulty is the effect of the changes to individual universities' research ratings between the two selectivity exercises in 1986 and 1989; the outcome has been to benefit universities at which a poor research department has been raised to a modest status at the expense of those which kept their best at the best. In short, there is evidence that some recent attempts to be selective have favoured the less good at the expense of the good, undermining the dual-support system in the process.

Some steps have also been taken to concentrate the deployment of research funds. Indeed, the funding of the dual support system in universities, but only in vestigial form in the polytechnics, is in itself a concentrating influence. But that implies that any decision to open up the dual-support system to the polytechnic sector without a major injection of extra money would be strongly anti-concentrating in impact.

Concentration

In reality, the concentration of research within the university sector has been under way for years. Some research topics are so expensive (or otherwise demanding) that only a few institutions are able to participate in them. Examples are astronomy (at least when it requires large telescopes) and space science. One response to the erosion of the dual-support system must be the further concentration of research. If we cannot afford to have an adequate dual support system across the whole of science and in all universities, we must ensure that it thrives in some research areas in good departments in some universities.

As concentration develops, research in particular areas of science will become even less evenly spread across the university system. I believe this must be accepted. Moreover, if the process is to be effective, the widening of the dual-support system to encompass the polytechnic sector needs to be sharply curtailed. It is reassuring that, in a recent statement, the Secretary of State has said that "it would be unacceptable to me for research funds to be distributed universally across both the present sectors."

But even though an effective dual-support system requires concentration, I do not believe that concentration should go so far that any single institution receives no research support from the funding council. Although there are many prestigious institutions in the United States that pursue only teaching and scholarship, I believe that Britain's future need of scientifically trained manpower requires the output from all our universities, and from some of our polytechnics as well.

But the impact of selectivity and concentration does require that we contemplate the emergence of a cohort of research universities' seeking to pursue world-class research across a broad range of subjects. We are already some way down that road. For example, 54 per cent of all the SERC's research grants now go to only 11 of our HEIs, all universities: Birmingham, Cambridge, Edinburgh, Glasgow, Imperial College, Leeds, Liverpool, Manchester, Oxford, Southampton and University College London (in alphabetical order).

One way to effect a sharp concentration of research in our HEIs would, of course, be to terminate the dual-support system and to transfer all the money now deployed through both its channels to the research councils. This would increase the funds deployed by the councils for direct research support from £240 million a year to £1,100 million. If the councils then solely funded high-class research, as judged by peer review, the effect would certainly be strongly concentrating.

A move in that direction has already been agreed. From August 1992, an annual sum of about £150 million will be transferred from UFC to the research councils. This is about 17 per cent of the funds currently allocated by UFC for research. With the transfer will come added responsibilities for the councils, particularly the requirement to fund some of the indirect costs associated with research that are now met by the universities from the funds they receive from the UFC.

How far could this trend safely go? If all the money for the support of research were transferred from UFC to the research councils, the likelihood of good researchers making a start on their res-

earch careers would be greatly reduced. Such people first come to light in the universities, and in their early development have little by way of established reputation with which to back their claims for research support. Although means by which the research councils could help such people could be devised, I believe it is far better that the task should be undertaken by the universities out of research money allocated by UFC.

Concentration may also well mean that an increasing number of bright potential researchers will emerge in university departments whose research performance is only average, or less. Some arrangements will have to be made to help such people rapidly to join departments where research is strong.

Need for change

A further reason why a substantial amount of research money should be left with the universities is that it is necessary to facilitate change. Departments with weak research need the opportunity to develop into strong research departments, while strong research departments do not automatically persist as such forever. I believe the universities (not the research councils) should have the prime task selectively and purposively to evolve the research strengths of their departments — using research money provided by UFC.

On the other side of the dual support system, not all research council funding of research should flow through a single council. Multiplicity of choice is vital: no single panel of assessors is gifted enough always to make the right decisions on what to support.

So, to broaden opportunities for research and thereby to improve morale, we need a multiplicity of funding sources forming a robust and effective dual support system concentrated on a restricted number of departments and universities carrying out excellent research. The questions then arise of how hard concentration and selectivity (particularly the former) should be driven, subject by subject and institution by institution. Certainly the research funds now available should be spread less thinly than they are.

These questions prompt that of where and how such decisions should be taken. The Advisory Board for the Research Councils (ABRC)? Or UFC? Who should be responsible for the overall strategy embodied in the dual support system? Clearly, neither ABRC nor UFC can do the job alone. (UFC, until recently, has shown little taste for becoming involved in questions of this kind at all.) I believe a combination of concern over how the dual-support system is cherished, how priorities are set and how research money is deployed effectively in HEIs points to the need for a much more research-focused structure. One clear need is for an

Advisory Body for Research (ABR) — not just for the research councils — and the other is for an effective research-focused structure within each HEI to further research and to ensure that research money is effectively deployed.

In my opinion, the second of these requirements points irresistibly to the need for graduate schools to promote research in at least those HEIs which are to be strong in research. I also believe that UFC should at least have a research committee to guide its policy. But ideally, I believe the combined UFC/Polytechnic and College Funding Council should be split into a Teaching Funding Council (TFC) and a Research Funding Council (RFC) (see figure, a corrected version of the one on page 203, *Nature* 19 September). Such a structure would allow cogent policy to be set, but still leave a necessary degree of autonomy with the HEIs.

An essential element of this arrangement would be that money intended for research (that is, allocated on research-based criteria) should be channelled exclusively through the graduate schools, which should be, legally, parts of their HEIs, but independently accountable within their institutions for the management of research. The research councils and UFC, if in agreement on this objective, could have a powerful influence on its attainment.

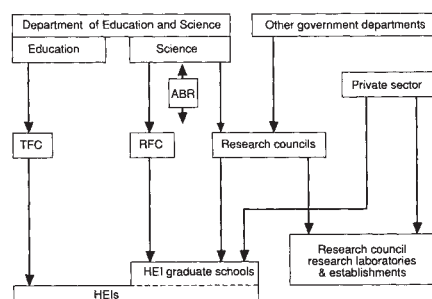
It is important that such structures are not intended to imply a detailed direction of research from above. The ABR would be concerned only with overall strategy and advice to the Secretary of State. Graduate Schools would aim only at the effective deployment of and accountability for resources. The content of research would be left, as now, to the bids made by individual researchers to the research councils, the charities and the HEIs themselves. ABR's influence would be exerted to deploy selectivity and concentration with advantage, and to strengthen dual support.

But would not this arrangement attack the sacred principle of the inseparability of good teaching at undergraduate level from research? This need not be the case. The existence of a graduate school need not prevent its researchers from teaching — but it should help to protect them from debilitating teaching loads.

If the strengthening of dual support, albeit in a more concentrated mode, is one vital element for improving opportunity and therefore morale, another is a greater continuity in the level of support available through the dual-support system. The past financial year, in particular, has seen a sharp cutback in the funds available, notably because of a disappointing level of funding through the research councils, especially SERC. (At SERC, the escalating costs of international commitments

has put the funding of the dual support system under even greater strain.) I believe there is a clear need for some device to smooth the flow of resources into research so that the current stop-go pattern of funding is avoided. There is nothing more damaging to morale than good work funded one year but not the next.

In summary, then, I believe the enhancement of opportunity by the strengthening of the dual-support system under the guidance of a more adequate research advisory and planning structure is long overdue. Without these changes, I cannot see research in our HEIs being really attractive to the gifted potential young scientists in Britain. The consequence will be a continued decline in our scientific standards and an impoverishment of our science base — both the research and the people. Something must be done, and soon.



So much for opportunity: what about remuneration? One characteristic of British researchers is, in general, their relative unconcern with pay. Opportunity to do good research is, I believe, their higher priority. But that does not apply to those who may be considering a life in research. And should those already committed be abused by taking advantage of their obsession with good science? The starting salaries of young scientists, and the levels of remuneration available to students who wish to do a PhD degree and thereby take the first steps in a research career, are a national disgrace. How many scientists would now consider starting on a research career at a stipend of £4,125 a year? A career in industry and commerce would provide a starting salary nearly twice as great. Even a postdoctoral research worker would on average be likely to join an HEI at a salary only 1.3 times that of a postman and only 10 per cent more than that of a policeman.

The government seems to see no electoral disadvantage in denying our research workers the rewards their intellectual gifts are due. I believe politicians should not be too cynical about the vote-winning potential of an issue such as this. Furthermore, one sees little evidence among our main competitors in Europe that their political leaders are as seemingly uninterested in these issues. Unless there is a strong and effective political commitment to support

the research base, Britain will fall even further behind in the quality of its research base and in its ability to be a figure of distinction among the top nations of Europe. The same comparison obtains with the United States.

I am not renowned for my calls on the Government for more money. Indeed I have spent a good deal of the past ten years trying to extract better value for money from the funds allocated to the universities and the research councils. And I have already made clear that there is more that can be done in that direction.

But that cannot absolve the Government from the need to inject more resources into the support of research under both legs of the dual-support system — UFC and the research councils. The sums necessary to enhance opportunity (particularly with concentration) need not be huge. A recurrent sum of £100 million a year would have an immense and immediate impact and could be deployed effectively. But if we are to retain our excellence in research, and provide the needed flow of highly qualified young people into research, it is major relative increases in remuneration that are needed. The cost of this could be around £130 million. Without a move in this direction, I believe the long-term survival of quality research in Britain is seriously at hazard.

There is only one person who, in the first instance, can do anything about this and the time is now ripe. The Secretary of State for Education and Science gives every indication of priding himself on his qualities as a minister. Let us see him deploy these qualities in a positive way to support the research base. Let him get the resources to provide the opportunity and the remuneration needed to make a decision by a person to go into a career in science no longer an act of self-immolation. This does not have to be done in one step, but a start must be made.

So, in conclusion, a strong science base urgently needs extra funding, primarily in the form of adequate pay for young scientists. But it also needs a greatly strengthened dual-support system. Ingredients for this are even greater selectivity and concentration; and management arrangements involving a more purposive attitude by the UFC, an integrated surveillance and policy formulation by an Advisory Body for Research (not an ABRC) and the channelling of resources to support research exclusively through graduate schools in our HEIs.

We still have immense quality in the researchers in our higher education institutions. They must be protected, for they have much to contribute to the future prosperity of our country. □

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NIH push for women's health

Heart disease, osteoporosis and cancer win a new claim on NIH's attention as political pressure in the United States drives women's issues to the top of the research agenda.

For a year now, the US National Institutes of Health (NIH) have had an Office of Research on Women's Health. When cardiologist Bernadine Healy became the first woman head of NIH earlier this year, she was quick to announce a major research initiative on women's health. Whatever her difficulties with congressman John Dingell over the NIH's fraud office, Healy will win universal praise from Congress for this politically correct stance on women. It is an interesting development.

A cornerstone of the initiative will be a \$500 million decade-long study of between 60,000 and 70,000 post-menopausal women who, by virtue of age, are at risk of having a heart attack, dying of cancer or becoming disabled by aged and brittle bones.

The study is being designed to assess the effects on women's health of diet, hormone-replacement therapy, calcium and vitamin D supplements, and exercise. The study will probably also cover the use of anti-oxidants, the relative value of detecting disease early and the prevention of smoking. It will be the largest epidemiological analysis of its kind ever undertaken in the United States.

Healy says this study of post-menopausal women will be but one of several initiatives coordinated through the Office of Research on Women's Health, which was created, before she became director, at the insistence of the Women's Health Caucus of the Congress, urged on by a group of women in science who have since formed the Society for the Advancement of Women's Health Research.

The premise underlying the movement is that research on men's health cannot be readily extended to women, and that research on women of one ethnic group cannot necessarily be applied to another. "Hence, research must take into account the cultural differences that occur among such diverse groups as African American, Hispanic, Native American and Asian women." The women's movement also places a good deal of faith in the belief that disease can be prevented by appropriate behaviour, implying that the behavioural and social sciences are as important as the traditional biomedical sciences in creating a new research agenda.

Women's health did not spring onto the political agenda just in the past year. The issue has been gaining momentum since the early 1980s, when the government appointed a task force whose recommendations, not surprisingly, sound very

much like those being made now. In response to previous calls for more research on women, NIH made an inventory of the research they supported in 1987, calculating that 13.5 per cent of their funds, or \$778 million, were spent on problems unique to women, while approximately 80 per cent went to basic research. That leaves less than 7 per cent for research on disorders unique to men.

Nevertheless, a case can be made that certain types of research have focused almost exclusively on men to the detriment of women. Heart disease is one. In the United States, more than 750,000 people a year die of heart disease — and 49 per cent are women. Yet the general impression is that heart disease is a killer of men, largely because men have heart attacks in middle age while most women are immune until they pass menopause. So the major clinical trials of beta-blockers, lipid-lowering agents, angioplasty and bypass surgery have been carried out almost exclusively with men.

Moreover, studies published in the *New England Journal of Medicine* (266, 221–230; 25 July 1991) show that physicians tend to treat heart disease in women less aggressively than in men unless or until a woman has an outright heart attack. Healy calls this the 'Yentl Syndrome' after the woman in Isaac Bashevis Singer's short story who had to dress up as a man in order to study the Talmud.

As a result, there are demands not only that women should routinely be included in clinical trials but also that gender differences be taken into account in designing protocols. A simple example is that of oestrogen therapy, now known to reduce the risk of heart disease in post-menopausal women by as much as 47 per cent. But an oestrogen trial 20 years ago yielded no evidence of benefit. (It was conducted in middle-aged men.) Now, the NIH have rewritten grant application forms, requiring that clinical trials include "an adequate number of women" unless the investigator provides a reason why there should not be that will pass muster with reviewers. (The Institute of Medicine has been asked to advise the NIH on inclusion or exclusion of women of child-bearing age.)

NIH's new-found emphasis on women is just one more example of how the institutes have for decades been pushed (or led, depending upon point of view) by political pressure. Special initiatives satisfy political customers and are, as often as

not, motivated as much by the desire to find a cure as by scientific opportunity. There are elements of both in the women's health initiative.

A recent three-day NIH workshop, convened to plan a research agenda for a decade and more, tells the tale. Very little was said about scientific opportunity but there was a lot of talk about the social and medical problems perceived to be particular to women. The meeting, intended to produce a genuine research plan, not surprisingly produced a laundry list of needs but no corresponding list of research projects that might yield solutions.

The first draft report of the proceedings (which needs further iteration) lists every conceivable aspect of a woman's life, from conception to death eight or nine decades later, as urgent matters for NIH research. It calls for research on the causes of teenage pregnancy, the effects of poverty on infants and the "dynamics that support a woman's decision to breastfeed". Depression, suicide and post-traumatic stress disorder in teenage girls are on the list, as are the increasing incidence of breast cancer in adult women and post-menopausal coronary disease.

Although light on proposals for research projects, the draft is replete with exhortations to change behaviour to prevent disease, sexually transmitted and cardiovascular alike. There is even a proposal to study ways of persuading young women to exercise so as to develop strong bones to ward off osteoporosis later in life. (It may be the first time NIH have been asked to get girls to take gym.)

It is vital that the relationship between behaviour and disease should be rigorously dissected to separate fad from wishful thinking from valid science. Many people, including an apparent majority at the women's health conference, place as much emphasis on behaviour as on medicine, a view that would predicate a change in the way NIH see their mission. This is entirely appropriate, provided it is accompanied by hard-eyed protocols for research in behavioural science that would advance the field.

What is missing from the draft are proposals for scientifically innovative ideas. The plan in Congress is to bring the National Institute of Mental Health back within the NIH fold to strengthen that tendency. But that will be good for women only if it is accompanied by first-rate science. That is the challenge.

Barbara J. Culliton

Schrödinger's catflap

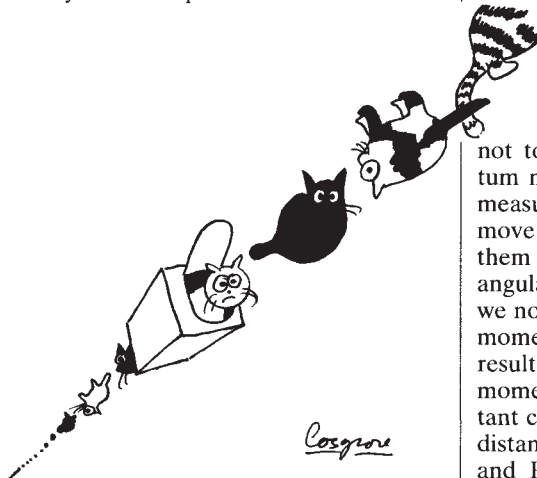
Ian Stewart

THE military and commercial use of ciphers has a lengthy history, but cryptography only became a branch of mathematics in the 1940s, when Claude Shannon invented communication theory. The main thrust of the mathematical approach was towards error detection and correction, and the methods were algebraic and combinatorial. The discovery of public key cryptosystems — in which methods for encrypting messages can be made public, but decryption is virtually impossible in the absence of a secret piece of information — introduced ideas about the efficiency of computational methods. It is possible to go beyond classical computation, however; and an emerging branch of the subject, known as quantum cryptography, does just that. Artur Ekert has now explained (*Phys. Rev. Lett.* **67**, 661–663; 1991) how ideas based upon the Einstein–Podolsky–Rosenberg (EPR) ‘paradox’ in quantum theory can be used to guarantee secure distribution of a cryptographic key. A forthcoming paper by Charles C. Bennett, François Bessette, Gilles Brassard, Louis Salvail and John Smolin, to appear in the *Journal of Cryptology*, involves very similar ideas, and also describes the history of the subject.

Quantum cryptography began in the late 1960s with unpublished work by Stephen Wiesner, who explained how quantum effects can in principle be used to manufacture banknotes immune to counterfeiting, and to transmit information securely. His work was eventually published (*Sigact News* **15**, 78–88; 1983) at Bennett’s instigation. The unfakeable banknote requires the ability to store a single polarized photon for several days without change of state. As Bennett *et al.* say: “Unfortunately the impact of the CRYPTO 82 conference had left most people under the impression that everything having to do with quantum cryptography was doomed from the start to being unrealistic”. However, in 1982 Bennett and Brassard realized that photons are not for storing information, but for transmitting it. Quantum versions of many mainstays of cryptography then appeared, including the self-winding reusable one-time pad, quantum coin-tossing, quantum oblivious transfer, zero-knowledge protocols and secure two-party computations. Here I’ll consider only the quantum key distribution channel.

Security of a cryptotext used to depend upon keeping the entire process of encryption and decryption secret. Nowadays, the encryption and decryption procedures can be public knowledge, pro-

vided only that a specific piece of information, the key, is kept secret from illegitimate users or eavesdroppers. The key can be any random string of digits. The danger with this approach is that all eggs are invested in one basket, the key. Before secure communication can begin, the key must be distributed securely to users. During the Second World War one rather amateur group of spies distributed new code keys by radio to possibly compromised agents, using the old (equally compromised) code. This is hardly an acceptable solution to the



problem; but more subtle distribution methods might also be open to eavesdropping.

Consider a specific example, an eavesdropper trying to monitor a communication channel, say by tapping a telephone line. Observations of any system disturb it and so leave traces. Legitimate users can try to guard against this by making their own measurements on the line, to detect the effects of tapping. However, the tappers will be safe provided they can make measurements that produce smaller disturbances than the legitimate users can detect. In classical (that is, nonquantum) mechanics, this kind of ‘arms race’ of increasingly sensitive measurements can go on indefinitely (“big bugs have little bugs” and so on).

In quantum mechanics it cannot, however, because of the Heisenberg uncertainty principle. Measurements of some quantities, however delicately conducted, may change other related quantities by significant and irreducible amounts. In the conventional interpretation, a quantum system exists in a superposition of possible states, until a measurement is made, ‘forcing’ it into a pure state. This view is dramatized by the unenviable fate of Schrödinger’s cat, which lives inside an impenetrable box,

and is alive if a particular particle has positive spin, dead if it has negative spin. Until the box is opened, the unfortunate beast is neither alive nor dead, but in a quantum superposition of the two states.

It is here that the EPR paradox, proposed in 1935, enters. The idea was simplified by David Bohm, and it is his version that I will describe. The proton is a particle with spin $\frac{1}{2}$. Its (spin) angular momentum, measured in any direction, is therefore $\pm\frac{1}{2}\hbar$, where $\hbar = h/2\pi$ and h is Planck’s constant. Begin with a system of two protons, very close together, of total angular momentum zero, a situation that can be realized in practice. If the angular momentum of one proton in a given direction is $\frac{1}{2}\hbar$, then that of the other must be $-\frac{1}{2}\hbar$; if that of the first is $-\frac{1}{2}\hbar$ then that of the second must be $\frac{1}{2}\hbar$. It is not necessary to measure these quantities for this relation to hold — indeed it is important

not to, because measurements in quantum mechanics disturb the system being measured. Assume that the protons move apart until the distance between them is large: the relations between the angular momenta will remain valid. If we now measure a component of angular momentum for one proton, then the result forces a definite state of angular momentum in that direction for its distant companion. This kind of ‘action at a distance’ seemed to Einstein, Podolsky and Rosenberg to be paradoxical, and they concluded that quantum mechanics must be an incomplete description of reality. But John Bell subsequently derived a theoretical relationship, Bell’s inequality, that permits experiments to test the EPR paradox. The results confirmed the predictions of quantum mechanics.

Ekert’s communication channel (that of Bennett *et al.* is similar) repeats the EPR model once per bit (binary digit’s worth) of transmitted message. It is a source that emits pairs of spin $\frac{1}{2}$ particles in opposite directions — that is, a box with a catflap at each end, which emits pairs of perfectly anti-correlated Schrödinger’s cats in a constant stream. If one cat in a given pair is alive, then the other is dead, but until their state is measured, we do not know which is which. At opposite ends of this axis are two time-honoured characters in the lore of cryptography, Alice and Bob, who represent the legitimate users of the channel. Their task is to extract from the sequence of EPR-linked particles a common key, and also to monitor the channel against eavesdropping.

To this end, they each measure components of angular momentum, in orientations which they select randomly and independently for each incoming

PLANT GENETICS

Abnormal developments

Elliot M. Meyerowitz

particle, thereby determining the conditions of their respective cats. Alice has the choice of measuring at angles of 0° , 45° or 90° in a plane perpendicular to the communication axis; Bob at angles of 45° , 90° or 135° . Each such measurement yields a result of $+1$ or -1 (in units of $\frac{1}{2}\hbar$), and potentially reveals one bit of information. If Alice and Bob measure angular momentum in identical orientations, then their results are totally anticorrelated: the state of Bob's cat is always the exact opposite to that of Alice's. But if they use different orientations, it can be shown that a particular combination S of correlations must be $-2\sqrt{2}$.

After transmission, Alice and Bob publicly announce the sequence of orientations they have chosen, and thus can privately divide their measurements into two groups: those for which they used the same orientation, and those for which they used different ones. They also announce the actual measurements they obtained in the second group, so that they both can compute the quantity S . If the channel has not been disturbed by an eavesdropper, the value should be $S = -2\sqrt{2}$. If that is the case, then the first group of perfectly anticorrelated measurements (whose values are known only to Alice and Bob) can be used to establish a common key.

The system is not secure to an eavesdropper who taps the channel only when Alice and Bob are measuring identical directions; but because they choose directions randomly, there is no way to achieve this without the services of an 'oracle' — and an oracle can defeat any system far more simply, just by intuiting the key. An eavesdropper might attempt to subvert the entire procedure by setting up a fake source that emits three particles rather than two, and intercepting the third stream of particles to see which way the cat jumps. Delayed measurements of those states, made after Alice and Bob announce their results to the world and in particular to each other, might reveal the key to the eavesdropper. Ekert conjectures that in fact there is no way to achieve this; and notes that in any case the eavesdropper runs into the 'banknote problem' of storing the particles without degradation of state until the announcement is made.

Experimental methods designed to test Bell's inequalities, such as those of A. Aspect, P. Grangier and G. Roger (*Phys. Rev. Lett.* **49**, 91; 1982) could be used to implement this scheme (over short distances) in a relatively practical manner. For quantum cryptography, Schrödinger's cat is out of the bag. $\square$

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MODERN genetic saturation analysis of embryonic pattern has arrived in the Plant Kingdom, as announced on page 402 of this issue¹ and in a complementary paper in *Development*². From analysis of 44,000 mutagenized lines of the laboratory weed *Arabidopsis thaliana* (the common wall cress), Jürgens and co-workers have extracted 250 strains in which the pattern of development that leads from zygote to seedling has been disrupted, in specific and repeatable ways. They estimate the number of zygotically active genes represented by this collection to be around 40, and that mutagenesis for such genes is near statistical saturation.

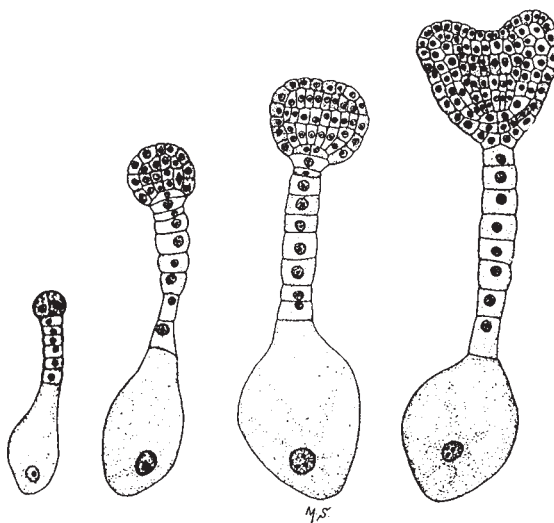
Embryo-lethal (non-germinating) lines are explicitly excluded from this count, and in this lies the originality of the approach. The authors obtained 25,000 embryo-lethal lines (representing an estimated 4,000 essential genes) but set them aside, as the majority are likely to be lines in which the primary defect is disruption of general cellular functions^{3,4}. By excluding the embryo-lethal mutants and analysing only the abnormal seedlings of mutants that are able to germinate, it was possible rapidly to identify mutants with profound and

meristem), central (hypocotyl) and basal (root). Homozygotes of the *gurke* (cucumber) mutant lack the apical region; *fackel* (torch) homozygotes have the cotyledons attached to the root, with no hypocotyl; *monopteros* homozygotes lack both hypocotyl and root. *gnom* homozygotes lack both apical and basal elements, causing the plant embryo to be a tiny cone or ball. There are pattern elements along the radial axis as well. Morphologically, the tissues on this axis are epidermis, ground tissue and vascular tissue. *knolle* (tuber) mutants appear to lack epidermis, while *keule* (club) has abnormal epidermal cells. The remaining mutations affect embryo shape, without eliminating any easily recognized pattern elements. An example is *mickey*, which has the effect on cotyledon size and shape implied by the name.

The pattern defects in the apical-basal class appear before the heart stage of embryogenesis; the radial phenotypes are evident in the globular stage (see figure). This makes it clear that pattern mutations that survive to the seedling stage can have their initial effects in the earliest events of embryogenesis, validating the criteria used in the mutant screen. The early phenotypes of the mutants also demonstrate that the genes identified have essential functions in the earliest stages of development. The nature of the early phenotypes (missing cell populations, or abnormal embryonic shape) strongly implies that the primary lesions are in early cell-division pattern.

Higher plants develop without cell migration, and with only modest changes in cell shape, so the primary determinant of plant form is a precisely controlled pattern of cell division. Because the departures from wild type shown by these mutations occur early in embryogenesis, when there are only a few dozen cells, it should not be difficult to find the exact initial defect or defects in each mutant. The key to understanding these

mutants is thus to find the exact departure from the normal pattern of cell division that occurs in each, and in so doing to define the relation between lineage pattern and differentiation in wild-type development. Molecular cloning of these genes will then lead us to the biochemical mechanisms that determine the time and plane of cell divisions. $\blacktriangleright$



Early developmental stages in *Capsella bursa-pastoris*, like *Arabidopsis thaliana* a small mustard, and with embryonic development almost identical to that of *Arabidopsis*. The drawings are of early (11-cell), globular- and heart-stage embryos (from ref. 11).

specific pattern defects.

The mutants fall into clear classes, with the nine best-studied loci giving mutant phenotypes of either apical-basal pattern deletions, radial pattern defects or specific shape changes. The deletions show that the plant embryo has three pattern elements along the vertical axis, apical (cotyledons and shoot apical

Spinal tap

FOLLOWING the linkage of the common neurodegenerative disease spinal muscular atrophy (SMA) to the long arm of chromosome 5 last year, efforts are underway to identify the abnormal gene(s). The most intriguing candidate to emerge thus far is described by L. L. Lien *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **88**, 7873–7876; 1991), who used antibodies to dystrophin to isolate related genes including that of microtubule-associated protein-1B, which encodes a large cytoskeletal protein highly expressed in the anterior-horn motor neurons affected in SMA. Tight linkage was found between the SMA locus and MAP-1B, but two small chronic SMA families possessed recombinants. At worst, then, MAP-1B is a very close marker for SMA; at best, given the possibility of nonallelic heterogeneity in the chronic disease, it may have a more direct involvement in some cases.

Suspended animation

WHEN matter and antimatter meet, the normal expectation is that they immediately annihilate one another. So it came as something of a surprise to T. Yamazaki *et al.* two years ago to find that negative kaons can survive as long as 10 ns after arriving and stopping in liquid helium, before being pulled electrostatically into the host atoms' positive nuclei. Yamazaki and colleagues have now repeated the experiment with antiprotons, which (unlike kaons) have an infinite natural lifetime when isolated from ordinary matter (M. Iwasaki *et al.* *Phys. Rev. Lett.* **67**, 1246–1249; 1991). Although 96 per cent of the incident antiprotons in their sample spiralled immediately into helium nuclei, the remainder enjoyed a kind of suspended animation of up to 15 μ s, held, apparently in some metastable quasiautomatic state, before meeting their inevitable doom.

On the height side

A WIDENING gulf in the healthiness of rich and poor in industrialized nations may be read into adult death rates, points out J. P. Mackenbach (*The Lancet* **338**, 764; 1991), but other indicators reveal a brighter picture. Comparison of two surveys carried out in Holland in 1964–66 and 1980 indicates that the disparity in height between children of different socioeconomic groups again narrowed, continuing a trend seen since the turn of the century; for example, whereas in boys aged 11–16 the difference was 29 mm a quarter of a century ago, in 1980 it was 14 mm. Use of height may be an indirect indicator says Mackenbach. But he hints that those who consider the welfare state to have been unsuccessful in health care, given the adult mortality data, might care to think again.

That the mutagenic approach used here resembles that employed a decade earlier in *Drosophila*, to powerful effect, is not surprising: Jürgens, the senior author of these papers, was a postdoctoral fellow in the group that performed the saturation of the *Drosophila* genome for embryo pattern mutations^{5,6}. The ready application of *Drosophila* genetics to *Arabidopsis* again confirms Whyte's foresight, 45 years ago, in calling *Arabidopsis* a "botanical *Drosophila*". Indeed, mutagenesis of this sort is even simpler in *Arabidopsis* than in *Drosophila*, because the plant can self-fertilize, allowing homozygosis of newly induced mutations in a single generation. This is only practical for sex-linked mutations in *Drosophila*.

The original detailed observations of embryonic cleavage patterns were made on plants⁸, using *Capsella*, a relative of *Arabidopsis*. These were later followed by similar observations in animals⁹. Indeed, some of the first interpretations of cleavage patterns in animal embryogenesis derived from earlier con-

clusions on plant cell-division patterns¹⁰. With the application of saturation genetics and molecular cloning methods to reveal the mechanisms of pattern formation in plant embryos, plants may regain their position as the primary source of knowledge about patterned cell divisions in multicellular organisms. □

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COSMOLOGY

Texture and cosmic structure

Joseph Silk and Roman Juszkiewicz

AMONG the fundamental questions of cosmology are how the original hot primordial soup created in the Big Bang collapsed to become the galaxies we see today and why these are distributed with the particular degree of order that they have. One solution, proposed¹ by Neil Turok in 1989 following an earlier suggestion by Davis², was the notion of 'texture', a kind of froth that pervades the Universe and which would have arisen during the first moments following the Big Bang. In a series of papers^{3–7}, Turok and colleagues now show that texture could produce the observed spectrum of galactic masses and their spatial distribution. Nevertheless, provocative questions remain to be answered concerning this explanation for large-scale structure.

Global texture is a relic of the phase transition that demarcated the end of the grand-unification era, during which the nuclear and electromagnetic forces behaved as different aspects of a single force. An energy field, the Higgs field, was responsible for the grand-unification process when newly created, very massive particles were present that carried the fundamental forces of nature, the nuclear, weak and strong, and the electromagnetic forces. As the Universe cooled below the grand-unification energy scale (about 10^{16} GeV), the uniform part of the Higgs field, occupying most of space, decayed, leaving behind fun-

damental particles such as quarks and gluons that carry the strong force. However, occasional non-uniformities in the Higgs field survived as topological defects of 'knots' that are infinitesimal ($\sim 10^{-26}$ cm) regions of relic energy density that cannot decay.

Defects

Texture is not the only topological defect that can be created by phase transitions in the early Universe. Zero-dimensional, point-like monopoles, one-dimensional strings and two-dimensional walls have all been studied, but none has been shown compellingly to lead to a successful theory of large-scale cosmological structure. But the non-uniform Higgs field represented by texture results in three-dimensional knots with an energy density that generates density perturbations with just the right large-scale distribution to be of interest to cosmologists. Once the horizon scale (the distance travelled by light since creation) becomes comparable to the radius of a texture, the texture collapses owing to the tension associated with the deformation of the Higgs field. As it collapses, it drags in with it all of the associated energy density.

When it reaches a size of about 10^{-30} cm, the knot unwinds itself, emitting a burst of weakly interacting massless particles known as Goldstone bosons. The energy density associated with the

texture, and also with the expanding spherical shell of bosons, pushes ordinary matter around, which then seeds the cosmic structure we see today. Immediately after the texture generating phase transition, the Universe is filled with texture knots of all different sizes which, when the horizon reaches their respective size, collapse. The ambient matter responds to produce density fluctuations that eventually collapse to form galaxies, clusters of galaxies, and structures on even larger scales, leaving behind a background sea of undetectable Goldstone bosons.

There is only one free parameter in the texture model, namely the value of the Higgs field at the moment of symmetry breaking, and which is inferred to be of the order of the grand unification (GUT) scale. This fixes the amplitude of primordial-density fluctuations at first horizon crossing, thereby generating a scale-invariant or 'Harrison-Zel'dovich' spectrum of fluctuations from which large-scale structure eventually develops. A unique feature of the texture model is that the resulting density fluctuations are highly non-random. Normally, theories of structure formation adopt gaussian (or Poisson) initial conditions. With texture, however, there is a greatly increased probability of finding a large-scale fluctuation. This non-gaussian nature of these non-linear objects results in a distinctive pattern on large scales. Rare fluctuations seed large-scale structure and, given the usual normalization to rms fluctuations in the observed galaxy counts on a scale of a few megaparsecs, texture generates more large-scale power than in the standard cold-dark-matter (CDM) cosmological model introduced by Davis *et al.*⁸.

Missing power

If this feature of the texture-seeded model is real, it will certainly be good news. The problem of the 'missing power' in the standard CDM model has been recognized for several years, since the discovery of large-scale streaming motions (for a recent review, see ref. 9). The CDM density fluctuations are too small to explain the fluctuations implied by the streaming¹⁰, as well as those estimated directly from IRAS galaxy counts¹¹, or implied by the Oxford APM galaxy correlation function¹². There have been several attempts to save the model and add large-scale power by introducing a cosmological constant¹³, or by lowering the biasing factor¹⁴, and hence increasing the initial fluctuations. The texture-seeded CDM approach, seen in this context, is yet another theoretical attempt to add power on large scales while preserving the small-scale successes of the standard model.

Several recent papers by Turok and

collaborators³⁻⁷ develop the implications of the texture model for cosmology. Gooding *et al.*³ find that the scaling solution for evolving textures results in a mass spectrum of galaxy halos that declines inversely with the comoving scale of the texture-induced fluctuations. This results in a mass-spectrum of halos proportional to $m^{-1/3}$, in good agreement with the luminosity function of smaller galaxies. Hydrodynamical and dissipative effects in this picture have been investigated by Cen *et al.*⁴. On larger scales, Park *et al.*⁵ have performed N-body simulations in a texture-seeded CDM model, and find galaxy correlations and large-scale streaming motions in reasonable accord with current observations.

Perhaps the most intriguing suggestion was in an article by Turok and Spergel that appeared recently in *Physical Review Letters*⁶. These authors compute the probability distribution for the relative mass density contrast, δ , in the global-texture model, and explicitly calculate the deviations from a gaussian distribution in terms of the skewness of the distribution. An excess of positive density fluctuations relative to those predicted from a gaussian distribution is found. As in all intrinsically non-gaussian fields, the skewness $\langle\delta^3\rangle$ in the texture model^{6,7} scales like the variance $\langle\delta^2\rangle$ raised to the power $3/2$. This scaling relation may well provide us with a critical test for distinguishing between texture-seeded models and the conventional picture of gravitational instability. It is of particular interest in view of the positive skewness reported in a recent IRAS-selected sample of galaxies¹⁰.

Does positive skewness imply that our Universe started from non-gaussian initial conditions? Not necessarily. In models with gaussian, small-amplitude primaeval fluctuations, the skewness is zero at early times, and it grows later as a result of non linear evolution. This asymmetry in the probability distribution develops because δ can grow indefinitely in regions where the initial $\delta > 0$, whereas in the voids it can never decrease below -1 . For weakly nonlinear perturbations, the magnitude of the skewness can be calculated from second-order perturbation theory (to first order, the skewness vanishes). In a flat universe (that is, one with the critical density, $\Omega = 1$, for gravitational closure), the third moment of the density field is $\langle\delta^3\rangle = S\langle\delta^2\rangle^2$, where $S = 3/7$. Since this result was obtained by Peebles more than 10 years ago¹⁵, the subject remained dormant, presumably because the existing observational data were too noisy on scales large enough to make perturbation theory applicable. The recent detection of positive skewness in

galaxy catalogues has renewed theorists' attention and several groups are now involved in efforts to understand the relationship between different moments of the density field and the time evolution of the probability distribution δ , expected in the gravitational instability picture.

Smoothing

The preliminary results of this research look very promising. To make theoretical predictions comparable to estimates from galaxy redshift surveys, one has to take into account the smoothing of the density field with a filter of a finite width and the mapping from position space to redshift space. Both of these procedures modify S , and the most significant correction, caused by the smoothing, introduces a weak dependence on the effective logarithmic slope $n = d \ln P / d \ln k$ of the power spectrum P of density fluctuations, measured for wavenumbers k , corresponding to the cutoff induced by the filter. For $\Omega = 1$ and a gaussian filter, the value of S changes from 3.5 to 3.1 when n changes from -1 to 0 ; and for a top hat filter, $S = 3/2 - 3/n$. Both the IRAS¹⁶ data and the APM¹⁷ data for $20h^{-1}$ Mpc to $2\pi/k \leq 100 h^{-1}$ Mpc, where h is the Hubble constant in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, appear to be consistent with n of about -1 . Moreover, it turns out that gravitationally induced skewness is remarkably insensitive to the exact value of the density parameter: for an open universe ($\Omega < 1$) and infinite resolution the skewness S is enhanced by an infinitesimal additional term, $3/7(\Omega^{0.03} - 1)$.

The weak dependence of S on uncertain parameters like Ω and the shape of the initial spectrum made skewness measurements an excellent tool to test gravitational instability models with gaussian initial conditions (rather than a specific power spectrum). The second and third moments can be (and in fact,

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have already been) determined from galaxy surveys. Since for small enough δ , S is expected to be constant in the conventional picture whereas the texture model predicts $S \sim \langle \delta^2 \rangle^{-1/2}$, the power of the test increases with decreasing variance, and hence our ability to detect departures from gaussian behaviour will improve with the size of the sample. The existing catalogues may actually

be already big enough to make the distinction. □

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SEQUENCE ANALYSIS

Spinning in hyperspace

Willie Taylor

ONE of the main problems in systematic analysis of biological sequence data is that there is simply too much of it: if the human (or other) genome programme is even partially successful, then the data we have is little more than a sampler. Any new method that will speed the sequence comparison task is therefore of great interest — providing sensitivity is not sacrificed to the extent that important similarities are missed. A welcome step in this direction has been made in *Journal of Molecular Biology* by M. van Heel¹, who describes a new family of powerful multivariate statistical sequence analyses in which a clever transposition of technique applies image analysis methods from electron microscopy to the analysis of sequence data.

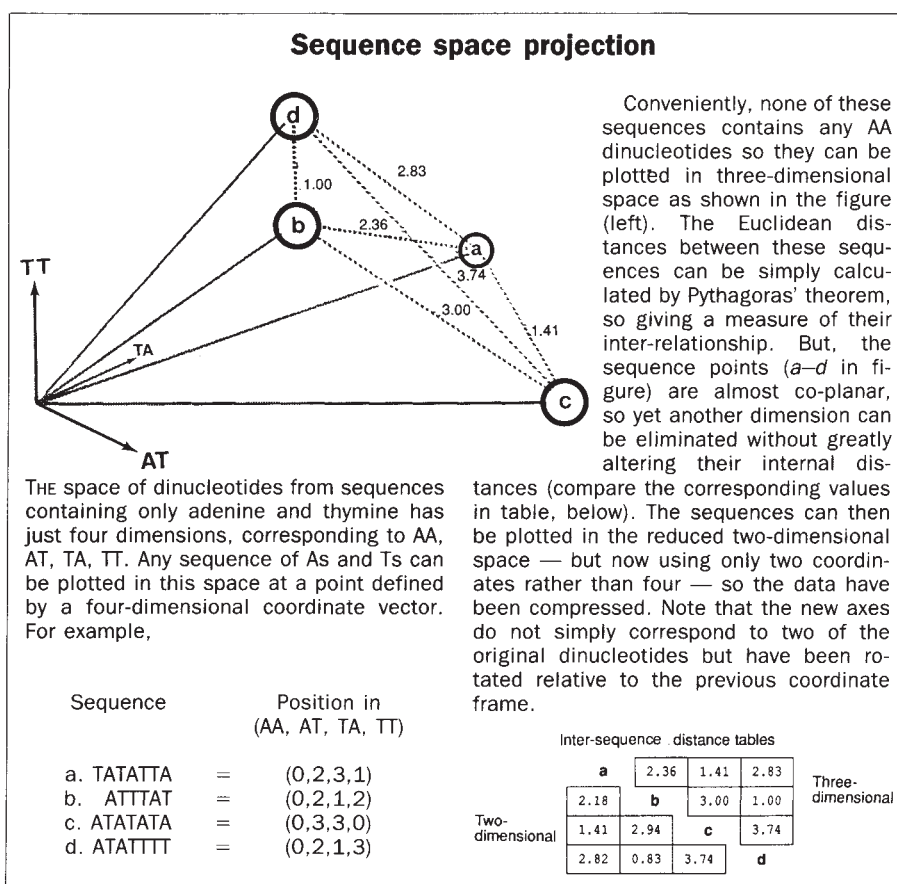
Over twenty years ago the mathematical technique known as 'dynamic programming' was established as the best way to compare and align two sequences². Unfortunately the dynamic programming algorithm is rather slow, and as the databanks grew, faster comparison methods were developed, based on identifying common short peptides between protein sequences³. (Although I refer to peptides and proteins, nucleic acid sequences are also implied.) For speed, the condition that these peptides form a consistent alignment was not required (because that would have forced a return to dynamic programming). Instead, the simpler check was made that there should be a reasonable number of matching peptides within close range of each other — that is, near a diagonal of the comparison matrix (or dot plot). Continuing this progression, a logical extension was to abolish completely the condition for any alignment. This method then simply measures the similarity of two sequences by the number of peptides they have in common⁴ and can be used for clustering large numbers of proteins into a partial order⁵. (A similar approach underlies the fast comparison program BLAST⁶.)

The work of van Heel takes us a step further along this progression: not only has he ignored alignment but also the

number of peptides considered has been reduced. The crux of his work is that this reduction is not carried out in a clumsy or arbitrary way, but by rigorous application of data compression methods. These techniques have been applied in guises such as factor analysis, principle component analysis, correspondence analysis, multidimensional scaling and distance geometry, to name a few. They have in common the ability to reduce complex (multivariate or multidimensional) data to a simpler form in such a way as to minimize loss of information. van Heel takes as his raw data the dipeptide compositions of proteins. Each protein sequence is described as a vector for which the value of each component (or coordinate) is the fre-

quency (in that sequence) of one of the 400 dipeptides (that is, all pairs of the twenty amino acids: AA, AC, AD . . . YV, YW, YY (in one-letter code)). Each protein can then be 'plotted' in this 400-dimensional space at the end of its dipeptide vector and the job of the statistical analysis is to consider all proteins in this space and find if any of the 400 dimensions can be discarded without much loss of information. As it can be difficult to visualize 400-dimensional spaces, a simpler example of the technique is illustrated in the Box. Such an analysis can be performed in any number of dimensions and in statistical terms reveals any co-variance in the data or in physical terms (treating the points as unit masses) defines the inertial axes.

Taking 10^4 proteins plotted in 400-dimensional dipeptide space, van Heel found that over three quarters of the dimensions could be dispensed with. In the resulting 96-dimensional space, he then measured the distance between all pairs of sequences and found that they cluster like stars into galaxies. Encouragingly, these constellations were familiar from previous (more labour-intensive) analyses, even down to the fine detail as revealed through the construction of phylogenetic trees. This is a surprising result, because throwing away data (through compression) might have been expected to blur this fine structure. It is possible, however, that the data com-



pression might actually help reveal the similarities between remotely related sequences by bringing together (in the same dimension) peptides that perform the same structural function; for example valine/isoleucine dipeptides (VV, VI, IV, II) might lie on a dimension that corresponds to positions in buried β strands. Overall, the results are preliminary and selective and the full sensitivity of the method will require time to evaluate, particularly in its relative performance against other peptide-based methods.

Having apparently retained sensitivity, the next question concerns the speed of the method. Two major calculations were performed: first, the large dimensional space is projected into a smaller one, and second, intersequence distances are measured in this projected space. The first step requires a massive matrix diagonalization which even with state-of-the-art algorithms (developed for image analysis) still requires a day on a reasonably fast workstation. For the second stage, although hard figures are difficult to extract from van Heel's results, the method is probably about as fast as the BLAST program (which can compare 1,000 sequences per second on a workstation), so although very rapid, it is not obviously better than present methods.

If no great advance in speed or sensitivity has been achieved, and given that similar methods (peptide measures and dimensional projection) are already known in sequence analysis, how are the techniques important? The answer is in their potential for further application. These have not been widely used on large volumes of data because the matrix calculations are slow. van Heel has shown that whole databanks can now be analysed and he suggests other directions for future research. He describes a neat spin-off from his main calculation: rather than plot proteins in peptide space, he turns his matrix on its side and plots peptides in protein space. The distances between peptides are then equivalent to a new peptide-exchange table that can be used (to good effect) in conventional sequence alignment. In summary, the method may not be the sought-after program for instant databank scanning but it provides us with a new tool, giving us a better view of the expanding space of sequences in which we live. □

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Only four possible solutions

Stuart Sutherland

THE investigation of the deficits produced in people by brain lesions has become a fashionable field of research partly because the patient and not the experimenter supplies the originality — the former produces the symptoms, the latter merely tests them — and also because the findings are often bizarre. There are, for example, brain-damaged patients whose ability to recognize inanimate objects is impaired while they are still perfectly well able to recognize living things; some patients can recognize abstract but not concrete words (and vice versa), others have difficulty only in recognizing fruits and vegetables, and so on. A common deficit is acalculia — a severe impairment in arithmetic ability. The investigation of one such case with some unusual features (L. Cipolotti, B. Butterworth and G. Denes; *Brain*, in the press) is marked by its thoroughness and by the ingenuity of the tests administered to pin down the nature of the disorder.

The patient was a 54-year-old woman who had suffered a stroke on the margin of the left frontal and parietal lobes. She recovered her linguistic abilities, except that she could not read or write; her intelligence at the time of testing was normal. Although she could not recognize single letters or arithmetic symbols such as '+' or '×', she could name road signs, the signs of the zodiac and playing cards. Her acalculia manifested itself as an inability to tell the time, dial a telephone number, recognize money, and even to give her own age.

When systematically tested, she could read the numerals 1–4 but no other (including zero). She could also recognize these numbers when spoken, but no other numbers. She could, with difficulty and using her fingers, count from 1 to 4. For pairs of numbers below 4, she knew which was the larger; she could also give the next cardinal number up to four. When shown cards containing randomly arranged black dots, she could say which had more dots, but only if at least one of the cards had four or fewer dots. When asked questions such as, "How many wheels does a car have?" or "How many days in a week?", she was always correct if the answer was four or less, but was vague or wrong if the right answer was above four. She could not remember sequences of more than four items, for example, the days of the week, months of the year, or the order of the alphabet, but she had no problems with the four seasons.

On the other hand, she performed normally on a whole set of other tasks

not involving numbers. For example, when shown a camel and a spider drawn as the same size, she knew the camel was in reality bigger. She could understand and manipulate transitive relations and could handle class inclusion: she was even right on that old chestnut, "If I have sixteen roses and six tulips, do I have more roses or more flowers?"

The authors have established with certainty that the only arithmetic concepts she had retained were 'one', 'two', 'three' and 'four'. She could even perform addition and subtraction with these numbers, not by remembering a result, such as $4-2=2$, but by counting after bending four fingers and then unbending two successively while counting 'one', 'two'. The authors rightly stress that her deficit was a central one: no matter whether tasks were presented visually or auditorily, the patient failed on all numbers except one to four.

What does it all mean? There is a faculty called subitizing, which is the direct perception without counting of the number of objects present in an array. The normal upper limit for this number is about seven. Perhaps the patient could only subitize up to four both in direct perception and in her imagination. Cipolotti *et al.* show that this is not the explanation: the patient was able to subitize up to only two items. They also consider whether her grasp of the numbers one to four was simply a result of these being the most common numbers. But as they rightly point out, if this were the case, she would be expected to become gradually worse with higher numbers, rather than having the sudden cut-off in her arithmetic ability.

There are other explanations that the authors do not consider. One is that the patient could use the fingers of one hand to count up to four and hence these numbers were preserved: admittedly, this leaves unexplained why she did not have the nous to use her thumb. A second, and perhaps the more likely, explanation is that the numbers one to four are those learned first. Later learning is laid down on top of previous learning. If therefore the connections that represent the numbers one to four were destroyed, all other number concepts would also be unavailable. But the situation is asymmetrical. Assuming the connections are laid down in the same part of the brain, the concepts learned first may survive, while others are lost. This fits with the patient's inability to deal with zero, which is a highly abstract concept and is presumably learned comparatively late in childhood. ►

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The authors have performed an ingenious and fascinating series of tests. Yet have their results forwarded our understanding of how the brain performs arithmetic? Regrettably, the answer has to be 'No', or perhaps 'Not yet', for the neuropsychologists would argue that one day another patient will come along with

REGULATORY PROTEINS

Ras and the *awd* couple

Rosamaria Ruggieri and Frank McCormick

ARE *ras* proteins regulated by nucleoside diphosphate kinases? This heretical proposition has re-surfaced with the discovery by Teng, Engele and Venkatesh (described on page 437 of this issue)¹ that the *prune* locus of *Drosophila melanogaster* has significant homology with Ras GTPase-activating proteins (GAPs). Loss-of-function mutations at the *prune* locus result in altered eye colour (*prune* instead of purple), but in combination with a second mutant gene, *awd*^{K-pn}, *prune* mutations are lethal². The *awd* locus encodes a nucleoside diphosphate (NDP) kinase, which catalyses transphosphorylation of GDP to GTP³. The interaction of NDP kinase with a GAP-like protein suggests that they are on the same pathway, and that this pathway includes a Ras-like GTPase. This possibility has emerged before in a very different context: the *awd* homologue *nm23* is a mammalian gene whose reduced expression may contribute to tumour metastasis as a result of the abnormal regulation of Ras-like proteins³.

Prevailing dogma

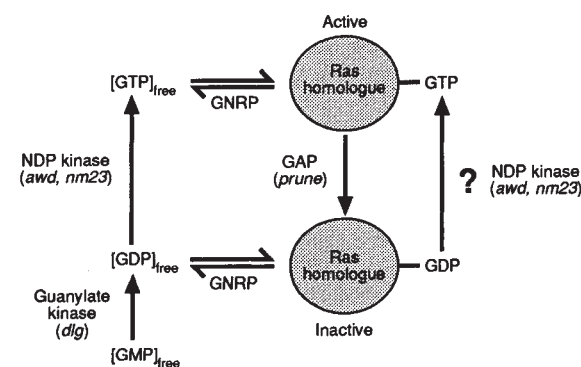
In the prevailing dogma, supported by abundant evidence from mammalian and yeast systems, Ras-like proteins are activated by exchange of bound GDP for GTP⁴ (see figure). Challenging this idea, NDP kinases could, theoretically, phosphorylate bound GDP to GTP directly, with no intermediate exchange event. GDP bound to tubulin is directly transphosphorylated by an NDP kinase in microtubule polymerization⁵, but so far we have no other convincing examples of the effects of NDP kinase on GTP-binding proteins, partly because of the difficulty in excluding local production of GTP from free GDP during the kinase reaction^{6,7}. On the other hand, NDP kinases do co-purify with a number of GTPases, including signalling G proteins and can be functionally coupled to these G proteins in cell-free systems⁸.

A less radical way in which NDP kinases might regulate Ras-like proteins could be to alter the composition of cellular guanine nucleotide pools so that the exchange process is affected. GDP/

a different form of acalculia, and when the deficits of the two are put together there will at last be light. □

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GTP exchange is thought to be executed in two steps. First, GDP is displaced. Subsequently, either GTP or GDP binds to the nucleotide-free protein. As the levels of GTP are normally much higher than GDP, the net result is binding of GTP preferentially, but this could



A possible scheme for the activation of Ras-like GTPases. GNRP, guanine nucleotide-releasing protein; GAP, GTPase-activating protein; NDP kinase, nucleoside diphosphate kinase.

change if the ratio of GTP to GDP is altered as a result of NDP kinase activity. Some evidence exists to support this possibility. For example, the rate of initiation of protein synthesis is regulated by GTPases distantly related to *ras* proteins (eIF-2 and others) and is sensitive to small changes in the GTP/GDP ratio⁹. Also, loss of a tumour suppressor gene of *Drosophila* (disks large gene, *dlg*, recently identified as guanylate kinase) causes neoplastic overgrowth of the imaginal disks, presumably by reducing GDP pools and increasing GTP/GDP ratios¹⁰. But these kinases may be subject to feedback inhibition by GTP and GDP (ref. 11), so their effects on GTP/GDP ratios *in vivo* are hard to assess.

Although levels of GTP in the cell are estimated to be much higher than GDP, these estimates, which are often in the millimolar range, do not distinguish between free and bound nucleotide. Measurements of free cytoplasmic GTP yield values between 50 and 150 μ M, at least in one cell type⁸. So, if the bulk of GTP in the cell is bound to or sequestered by proteins in microcompartments,

the local ratio GTP/GDP, which is controlled by NDP kinase and guanylate kinase, may have a significant impact on the nucleotide supply in the immediate vicinity of the GTPase protein. If microcompartments of GTP and GDP exist, this could account for the biochemical diversity of NDP kinases and their effects on certain GTPases, whose cellular distribution is compartmentalized. Likewise, the guanylate kinase present in septate junctions in epithelial cells¹⁰ might have a localized function relating to specific GTPases at these sites.

New evidence

Teng and co-workers¹ now provide new evidence connecting NDP kinase and a Ras-like GTPase. Mutations in the *prune* locus reduce the activity of GTP cyclohydrolase, the first enzyme of the pathway of GTP metabolism that leads to the

formation of pteridines, which determine the eye colour in *Drosophila*. But *prune* is not itself the structural gene for GTP cyclohydrolase: homology between the *prune* protein and the catalytic domain of GAP suggests that it is connected with a Ras-like GTPase involved in regulating pteridine synthesis.

How does this putative function of *prune* explain the lethal interaction between the *prune* and the *awd*^{K-pn} mutations? An attractive model proposed by Teng and co-workers¹ (see figure)

casts these two proteins as different regulatory elements of the same GTPase. Whereas the *prune* mutation causes a loss or reduction of function, *awd*^{K-pn} is a missense mutation which might confer a new function on the *awd* protein (loss of feedback inhibition by GTP, for example). The activity of GTPases can be regulated at two critical points, namely GTP hydrolysis and the renewal of GTP on the GTPase during signal activation. One of the functions of GAP is to inhibit Ras activity by stimulating hydrolysis of bound GTP to GDP, so the *prune* mutation alone could upregulate the pathway mediated by this Ras-like GTPase, leading to altered pteridine synthesis and to the *prune* eye colour.

Null mutations of *awd* are lethal but the *awd*^{K-pn} mutation on its own has no phenotype, so the alteration probably does not deprive the NDP kinase of its function and may even upregulate its activity. If the *awd*^{K-pn} mutation stimulates the Ras-like protein by increasing its association with GTP, the effect could be counteracted by a functional GAP (*prune*). But a combination of hyper-

active NDP kinase (*awd*^{K-pn}) and loss of GAP could lead to a level of GTP binding with lethal effects. This model raises the question of how many GTPases are likely to be targeted by hyperactive *awd* NDP kinase. The fact that *awd* null mutations cause loss of more than 98 per cent of total NDP kinase activity in *Drosophila* larvae¹² implies that alterations in this activity may greatly affect the total cellular GTP pool. The effects of *awd* during development might therefore be exerted on many GTPases. The *awd* null mutation causes abnormally condensed chromosomes to appear, perhaps as a result of defective phosphorylation of GDP bound to tubulin, which reduces polymerization of spindle microtubules¹², but the effect could equally well be attributed to loss of function of Ras-like GTPases that regulate chromosome condensation, like the yeast TC4 homologue¹³.

To substantiate this model, it will be necessary to prove that the *prune* product is indeed a GAP for a Ras-like GTPase. As Teng *et al.* predict, this GTPase may be isolated as a second site suppressor mutation of the *prune awd*^{K-pn} lethality, or as an activated mutation (equivalent to the Val-12 mutation in Ras) that gives a *prune*-like phenotype. In addition, it will be crucial to show that the *awd*^{K-pn} mutation hyperactivates *awd* NDP kinase.

As more NDP kinases come to light, it will become clear whether they operate specifically, locally, or in a general fashion, and whether they play critical roles in the regulation of Ras-like GTPases, as suggested by the *awd-prune* connection. In any case, understanding the role of NDP kinases and other enzymes involved in GTP/GDP metabolism will have broad implications for researchers working in many areas of cellular regulation. □

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Aiming the molecular arrow

Steven Stolte

ON page 412 of this issue¹ two researchers at Harvard University describe the application of a technique that is likely to have considerable impact on the study of the effect of molecular orientation on chemical reactivity. Building upon their own work and that of a group at the University of Bielefeld, Friedrich and Herschbach show that diatomic molecules can be oriented in an electric field, the field overcoming the natural randomizing motions of the molecules and restricting them to pendular oscillations. The molecule they worked upon is iodine monochloride (ICl) but the technique will be applicable to a much wider range of species.

Knowledge of how the orientation of an attacking molecule affects its reactivity is of central importance in investigating the elementary mechanisms of chemical reactions, where in a concerted way new bonds are formed and old bonds are broken². The prerequisite for studying such steric effects is the preparation of a beam of impinging reactant molecules for which one has control over which of a molecule's ends will be preferentially pointing towards the other reactant species. In the past such a beam could be produced only by filtering its oriented rotational states through an inhomogeneous electric deflection field. But oriented rotational states can be obtained in this way only for free rotating symmetric-top molecules, whose spinning axes will point preferentially along the direction of the (weak) electric guiding field^{3–5}.

Recently, the Bielefeld team (Loesch

and Remscheid)⁶ and that at Harvard⁷ independently started to develop an alternative method of orienting molecules by constraining their rotation by a strong electric field. This method avoids the cumbersome use of a state-selective focusing field and is not restricted to symmetric-top molecules. As a first step the Bielefeld group succeeded in investigating the orientational dependence of the benchmark reaction $K + CH_3I \rightarrow KI + CH_3$ (refs 6, 8). A second achievement is now described by the Harvard group, who in their experiments on ICl show that laser spectroscopy can be used to determine the specific orientation induced by the electric field for each individual rotational state.

Orienting molecules in a strong electric field is actually an old idea, and the Kerr effect (by which the double refraction of a liquid induced by an electric field can be studied) relates to this type of orientation. In liquids, where the unhindered rotational motion of a molecule is restricted by neighbouring molecules, an average orientation can be induced by applying an electric field. Its strength follows directly from the Boltzmann distribution⁹: $\langle \cos \gamma \rangle = x/3$ with x being the ratio of the electrical dipole energy to the thermal energy, $\mu E/kT$. Here γ is the angle between the molecular axis and the electric field E , μ the electric dipole moment of the molecule, k the Boltzmann constant and T the temperature. Angular brackets here denote the average (expectation) value. Not until 1972 was molecular orientation in liquids by a strong electric field quantitatively elucidated by measuring the influences of the alignment on the NMR spectrum¹⁰. In spite of the large fields applied ($E = 50$ kV cm⁻¹), however, the attainable orientation (x of up to 10^{-2}) remained small.

As Stern pointed out long ago, complications arise in the rarefied medium of a molecular beam, where the unhindered rotational tumbling of the molecular axis counteracts the orienting force of the applied electric field. The rotational states with the lowest rotational energy will carry out a pendulum-like motion, by which means their negative ends avoid pointing towards the negative electrode. For states with higher rotational energy a pinwheel motion

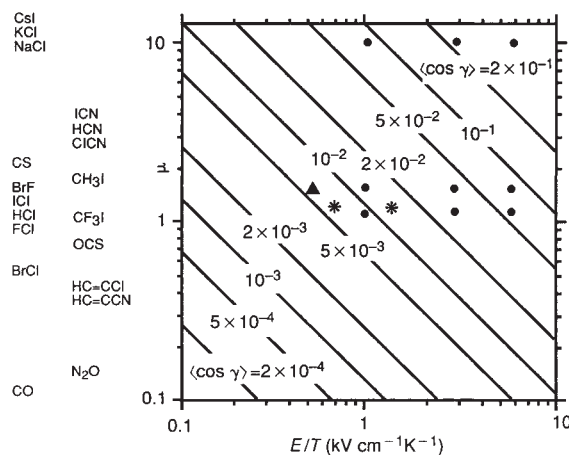


FIG. 1 Nomogram for the averaged orientation $\langle \cos \gamma \rangle$ as a function of the ratio of the electric field strength E (kV cm⁻¹) to rotational temperature T (K) and the dipole moment μ (debye). Values of μ for typical molecules are indicated at left. The symbol ● corresponds to calculations⁶, ▲ to the reactive scattering experiment^{6,8} and * to spectroscopic measurements¹.

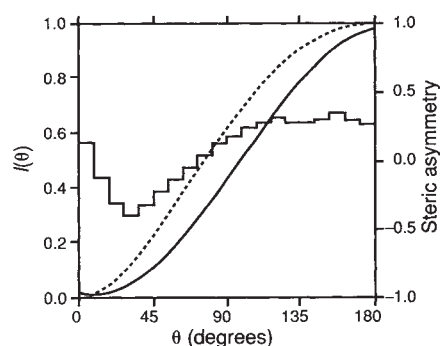


FIG. 2 Angular distribution of the measured (bold line) and predicted (stepped line) steric asymmetry and KI product intensity $I(\theta)$ (dashed line) for $\text{CH}_3\text{I} + \text{K} \rightarrow \text{KI} + \text{CH}_3$ as a function of the scattering angle θ for an incoming K velocity corresponding to a collision energy of 0.78 eV (ref. 8).

will occur, in which, owing to the deceleration caused by reaching the top of the energy barrier, the negative end of the molecule will spend most of its time pointing towards the negative field electrode. Moreover the rotating molecule also experiences a torque which tends to bend the plane of rotation such that the negative end of the molecule will point away from the negative electrode.

Calculation of the remaining amount of orientation, $\langle \cos \gamma \rangle$, is difficult because one needs to compute both the Stark effect and the eigenstates^{11,12} for a large number of rotational states of known occupation. This calculation, which indeed leads to a near cancellation of orientational effects, was carried out by Loesch and Remscheid⁶ for some simple molecules (KI, CH_3I and ICl) assumed to be cooled rotationally ($T = 5\text{--}30\text{ K}$) by expansion through a supersonic nozzle. Figure 1 shows the E/T values for which $\langle \cos \gamma \rangle$ was calculated. With an accuracy of about ± 20 per cent the results for small orientations can be expressed empirically as $\langle \cos \gamma \rangle = 0.3x$, which is about equal to the Boltzmann value.

Fortunately, the Bielefeld team was not discouraged by the weakness of the calculated alignment ($\langle \cos \gamma \rangle = 0.0063$) for CH_3I (resulting from their conditions of T of about 31 K and E of 16 kV cm^{-1}), and continued their attempt to measure the steric effect for $\text{K} + \text{CH}_3\text{I} \rightarrow \text{KI} + \text{CH}_3$. Indeed their superb experimental technique permitted a direct measurement of the orientational effect upon the angular recoil of the KI product. Figure 2 shows that scattered intensity of the KI product $I(\theta)$ is peaked in the backscattering direction $\theta = 180^\circ$. In other words, the product is ejected preferentially in a direction opposite to that of the incoming K reactant. Interestingly, the steric asymmetry (the difference in reactivity when K approaches towards the I atom or towards the CH_3 group) mimics the shape of $I(\theta)$. It

reaches its maximum value of 1 (I-end reacts only) for $\theta = 180^\circ$ and its minimal value -1 for $\theta = 0^\circ$ (CH_3 -end reacts only). The measurements of Loesch and Remscheid suggest that the KI product is ejected along the direction of the incoming CH_3I axis. That this type of information cannot be obtained from other data is exemplified by the very different steric asymmetry predicted from calculations⁸ using an empirical potential surface designed to account for all earlier observations¹³.

The paper in this issue¹ addresses the complementary problem of controlling the field-induced orientation. Typically the rotational-state populations within a nozzle beam are only approximately thermal. Friedrich and Herschbach demonstrate on ICl (see Fig. 1) that laser-induced fluorescence can be used to detect the orientation induced by the electric field for a single rotational state. The observed field-induced violation of the standard selection rules of spectroscopic transitions provides a quantitative characterization of the reactant orientation achieved.

The Bielefeld and Harvard teams have already shown⁶⁻⁸ that the scepticism about the possibility of producing oriented molecule beams with strong electric fields was misplaced. Although the net orientation that has been achieved is small ($\langle \cos \gamma \rangle$ of up to 0.01), appreciable improvements can be expected with lower temperatures or stronger fields (E/T up to 10, see Fig. 1). The demonstration of the practicability of molecular orientation for molecules other than symmetric tops now promises experimental insights into the mechanism not only of chemical reactions, but also of a growing number of other molecular collision processes for which the approach angle of the molecular axis is important. □

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Wings of the wind

LAST week Daedalus described the 'Ramcraft', a sea-going hovercraft whose air-cushion is pressurized by its own forward motion. Its hull is suspended inside a light, broad, parachute-like envelope whose forward facing 'mouth' scoops in the air. Its only contact with the water is the shaft carrying the propeller that drives it along.

Daedalus is now taking this elegant concept to its ultimate conclusion. His novel 'Windhover' is a wind-powered and wind-inflated hovercraft with no propeller at all. At first sight, this seems impossible. A sailing vessel exploits the differential motion between wind and sea. Deprived of water-contact, it would simply accelerate helplessly downwind until it reached wind-velocity; it would then feel no wind force, either of propulsion or lift. It needs a keel, both to define a direction of easy motion and to establish a load against which the force of the wind can usefully act.

But a light and speedy surface-skimmer like the Windhover would be fatally slowed by the viscous drag of a conventional keel. Daedalus plans to provide it instead with a line of thin disks mounted in good bearings. As the craft skims over the water, the disks project below it; each dips a little of its periphery into the water, and rotates freely in consequence. Thus its wetted surface is almost stationary with respect to the water, and imposes only a tiny fraction of the drag of a normal keel. More cunning still, each disk can also be pivoted vertically, like a rudder. As well as providing steering, this lets the craft twist at any angle to its direction of travel. It can even run fully sideways, with its disks lined up coaxially like those of a disk-harrow.

This subtle arrangement provides the Windhover with lift and thrust in any wind. By pivoting the disks, the whole craft can be rotated about its direction of travel. Thus the air-scoop of its overarching envelope can be kept facing the wind, maintaining the air-cushion pressure which keeps the craft hovering. To provide thrust, secondary openings can be made in the envelope through which the trapped air emerges in jet fashion. The envelope thus redirects the wind, in much the same fashion as a conventional aerofoil sail.

In a lively wind, the interactions between lift and thrust and keel-force will be so complex and rapid that the Windhover will need elaborate computer-control. But it should then prove wonderfully fast and flexible, outpacing even the fastest yachts with its low drag and amazing turning capacity. Its wheel-like keel-disks will even enable it to make good progress over land. DAVID JONES

Anti-cell antibody in macaques

SIR — Infection of macaques by simian immunodeficiency viruses (SIV) leads to a disease similar to that produced by HIV in man. This simian system is widely used in the United States and Europe as a model for the development of vaccines against AIDS. There are several reports¹⁻⁴ that inactivated vaccines completely protect against intravenous challenge with homologous SIV. It is widely assumed that, as with other viral vaccines, protection is mediated by immune responses to viral antigens in these vaccines. But, although the vaccines do induce high titres of antibodies to SIV, our attempts to correlate protection with titres of virus-neutralizing antibody or, indeed, any anti-SIV antibodies (T. Corcoran *et al.* and P. S. *et al.*, in preparation) have been uniformly unsuccessful. Similar observations have been reported by others^{1,3,5} and were discussed in News and Views⁶.

We have demonstrated that inactivated purified virions or fixed SIV-infected cells are protective. During experiments to define the protective components in these vaccines and the minimum dose schedule, four macaques (*Macaca fascicularis*) were vaccinated with inactivated SIV-infected C8166 cells (group A) and four with uninfected cells (group B) at 0 and 4 weeks using Quil A as adjuvant. These animals were challenged at 6 weeks with 10 MID₅₀ of SIV intravenously. Three macaques in group A and two in group B were protected.

In view of this surprising result, the five protected animals were revaccinated with their respective vaccines at week 27 and rechallenged at week 29. Two of three in group A and one of two in group B were protected. In these experiments and on the nine other occasions when this challenge virus was used, four of four unvaccinated control macaques became infected. Thus, there was no significant difference in the protection induced by the two vaccines.

The uninfected cell vaccine did not induce antibodies to SIV that could be detected by ELISA or immunoblotting. As expected, all vaccinated animals made antibody against C8166 cells which was measured by ELISA on the day of challenge. But we did not expect to discover that the mean titre of the eight animals protected against challenge was tenfold higher than the mean of five animals which became infected, and that this difference was highly significant. The mean titre $\pm$ standard error of protected group is $\log_{10} 3.49 \pm 0.07$ and of the infected group was $\log_{10} 2.42 \pm 0.08$, giving $P < 0.0001$ by *t*-test.

In the light of this finding we re-examined sera from animals vaccinated

with gradient- or column-purified virus as well as those given SIV-infected cells. Nine became infected following challenge and forty were protected. The mean titre of antibody to C8166 cells on the day of challenge in the protected group was $\log_{10} 3.39 \pm 0.09$ and in the unprotected group was $\log_{10} 2.39 \pm 0.10$, $P < 0.0001$. Certain of the animals vaccinated with purified virus had higher titres to C8166 cells than those vaccinated with fixed SIV-infected cells. Similar results were obtained when CEM-4 cells were used as antigen and surface fluorescence was measured by flow cytometry; mean titre of protected animals was $\log_{10} 3.67 \pm 0.12$, and of the unprotected groups was 1.75 ± 0.09 , $P < 0.0001$.

We are performing definitive experiments to confirm these observations, to determine the full extent of protection mediated by antibody to cellular components of the vaccine or challenge virus, and to define more specifically the target for this antibody. Our findings do not, of course, exclude the possibility of a virus-specific component in protection, and studies with recombinant antigens will eventually clarify this mechanism of immunity. In the meantime, we wish to publish our unexpected findings quickly so that others working with SIV in macaques or HIV-1 in chimpanzees can evaluate their results in the light of this information.

Whatever the outcome, experiments in animal models have demonstrated that vaccines induce complete protection *in vivo* against challenge viruses which induce AIDS-like disease; our results highlight the importance of the SIV-macaque model in which protective mechanisms can be examined. We are not aware that antibody against cell components has been shown to protect *in vivo* against any other type of virus infection. Our results, if confirmed, may reveal another unique property of immunodeficiency viruses which requires explanation and may profoundly affect our understanding of such viruses and the way we strive to control them.

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Red Rectangle emission

SIR — P. J. Sarre in Scientific Correspondence¹ has drawn attention to the near correspondence in wavelength between a group of diffuse interstellar absorption bands and some of the discrete emission features observed in the Red Rectangle², a coincidence that I have independently noted³. Sarre points out that the profiles of the emission features at wavelengths $\lambda\lambda 5,799$, $5,855$ and $6,615$ Å are typical of band heads formed by the contours of unresolved R-branch molecular rotational lines. I suggest that the more symmetric emission features at $\lambda\lambda 5,828$ and $5,880$ Å, and a shoulder on the red wing of the $\lambda 6,615$ feature^{2,4}, correspond in each case to the blended contours of the Q- and P-branch rotational lines.

For a given kinetic temperature, T , and a Boltzmann population of rotational energy levels⁵, the energy separation of the strongest R- and P-branch rotational lines is given by $(8kTB'/hc)^{1/2}$, where B' is the rotational constant of the molecule in the excited electronic state. The measured separation of a given pair of contour peaks therefore provides an estimate of B' , if the P-branch contour dominates the redward emission feature.

For a nebular temperature in the range 3,000–7,000 kelvin, $B' \sim 0.45$ – 0.20 cm⁻¹ for the $\lambda\lambda 5,799/5,828$ $\lambda\lambda 5,849/5,880$ pairs of contours; for the $\lambda 6,615$ feature and its shoulder, $B' \sim 0.10$ – 0.14 cm⁻¹ over this range of temperatures. Such rotational constants are typical of carbon-chain molecules of about three to seven atoms in length, or small polycyclic aromatic hydrocarbons containing between one and three rings. These values certainly exclude C₆₀ (as discussed by Sarre) and large polycyclic aromatic hydrocarbons as candidate molecules for the emission features.

The suggestion that carbon-chain molecules exist in the Red Rectangle is not new². I have argued elsewhere³ that the abundance of carbon-chain molecules and small graphite flakes in the diffuse features.

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Coronavirus motif

SIR — I report the presence of a leucine zipper motif at the carboxyl end of the spike (S) glycoprotein, a transmembrane protein of coronaviruses. All the coronavirus S proteins whose sequences are known — transmissible gastroenteritis virus (TGEV) FS772/70 (residues 1,342–1,377), feline infectious peritonitis virus (FIPV) 79–1146 (1345–1380), mouse hepatitis virus (MHV) A59 (1217–1252), MHV JHM (1128–1163), human coronavirus (HCV) 229E (1,067–1,102), bovine coronavirus (BCV) Mebus (1,266–1,294) and infectious bronchitis virus (IBV) Beaudette (1,058–1,079) — contain a

leucine-zipper motif terminating 10 amino-acid residues upstream of the conserved KWP motif preceding the transmembrane domain.

The length of the leucine zippers range from three heptad repeats, as identified for the F glycoprotein of paramyxoviruses, to five heptad repeats. The observation that all coronavirus S proteins sequenced so far contain the leucine-zipper motif 10 amino-acid residues from the transmembrane domain may imply some function of the motif in the dimerization of the S polypeptides.

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Virus	N° of amino acid residues	Leucine Zipper motif							Transmembrane Domain
		*	*	*	*	*	*	*	
TGEV FS772/70	1149	1341	-	NLTGEIDDLFEPRSEKLENTTVLAILIDNINNTLVNLEWLNRIETTVKWPVWLLIGLVV	-	1401			
PRCV 86/137004	1225	1117	-	NLTGEIDDLFEPRSEKLENTTVLAILIDNINNTLVNLEWLNRIETTVKWPVWLLIGLVV	-	1177			
FIPV 79-1146	1452	1344	-	NLTGEIDDLFEPRSEKLENTTVLAILIDNINNTLVNLEWLNRIETTVKWPVWLLIGLVV	-	1404			
MHV A59	1325	1216	-	DLSDLFKLVNLTFTYEMNRIQDAIKKLNESYINLKEVGTETMYKWPVWLLIGLAG	-	1276			
MHV JHM	1235	1127	-	DLSDLFKLVNLTFTYEMNRIQDAIKKLNESYINLKEVGTETMYKWPVWLLIGLAG	-	1187			
HCV 229E	1173	1066	-	NLTSEISTLENKSAELNYTVQKQLIDNINNTLVNLEWLNRIETTVKWPVWLLIGLVV	-	1126			
BCV Mebus	1363	1265	-	YINVTFLDLQDENRLOKAIKLINQSYINLKDICTETTYKWPVWLLIGFAG	-	1318			
IBV Beaudette	1162	1057	-	DIDSEIDRIQGVIGQINDSLIDLEKLSILKTYIKWPVWLLIGFAG	-	1103			

Comparison of the leucine-zipper motifs found in the different coronavirus S proteins. The KWP motif preceding the potential transmembrane domain and the transmembrane domains are also shown. The repeated leucine or isoleucine residues are marked with an asterisk. PRCV, porcine respiratory coronavirus.

Biocontrol risks

SIR — Hochberg and Waage suggested in their News and Views article¹ that some new genetically modified insect viruses will be acceptable as biological control agents because they have "highly restricted host ranges". There is widespread agreement that specific biological control agents are much to be preferred, on environmental grounds, over chemical pesticides. But the dangers of non-specific biocontrols are great, and much damage has resulted from their use².

How specific are these genetically modified organisms? They are derived from the *Autographa californica* nuclear polyhedrosis virus (AcNPV), a baculovirus, which has a wide and sporadic host range in the lepidoptera. There are around 2,500 species of lepidoptera in Britain³, and of course many times more elsewhere. The records of host range of this virus⁴⁻⁶, based on a small fraction of the known species, show that, of twelve superfamilies tested, eight apparently contain 'permissive' species (species killed by fewer virus polyhedra than are produced by one dead caterpillar). With this and other baculoviruses⁷ one species in a genus may be permissive, others resistant and the LD50s vary markedly between different permissive species, without apparent regard for taxonomy.

The superfamilies known to have permissive species are the Gelichioidea, Pyraloidea, Papilionoidea, Sphingoidea and Noctuoidea: the Bombycoidea, Geometroidea and Yponomeutoidea may have them, but this needs confirmation by DNA analysis. It seems that 5–10 per cent of British lepidoptera are permissive for AcNPV, a non-native virus, putting 125–250 species at risk, including some of great conservation value.

The two new genetically modified organisms^{8,9}, like others derived from the same virus¹⁰, may have host ranges slightly different from that of the wild type. But unless they can be further engineered to be absolutely specific for a known set of (pest) species, it is difficult to see that they could be used safely in an uncontrolled way in the field. Would not the risk assessment required under EC Directive 90/220, for instance, indicate that they are undesirable?

Hochberg and Waage¹ also note that "disabling engineered viruses so that they do not persist has some appeal". Removing the polyhedrin gene to produce a non-occluded virus^{5,10} reduces both persistence and, to a small extent, the host range; neither of the two new genetically modified organisms has apparently been modified in this way.

Research into the molecular and other

bases of host specificity is likely to be a *sine qua non* for the successful, much-desired, replacement of chemical control agents for insects by viral ones.

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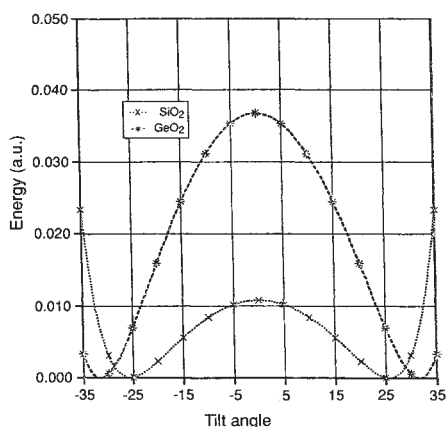
Ionicity in silica

SIR — Kramer *et al.*¹ suggest that a change in ionicity is responsible for the transition from the α to the β phase in silica. Their *ab initio* force-field method indicates that an increase by about 0.1 atomic units of the net charge on silicon stabilizes the β structure of quartz and cristobalite with respect to the α structures, implying that the former correspond to global minima of the Born–Oppenheimer potential-energy surface. Our recent Hartree–Fock calculations² on the quartz structures of SiO₂ and GeO₂ come to opposite conclusions with respect to both the magnitude and the direction of the ionicity effect.

Our calculations were performed at a level of accuracy similar to that of the cluster calculations used to derive the force field of ref. 1. We used the $P3_21$ space group for both the α and the β structures. In α quartz there are two equivalent twinned configurations, α_1 and α_2 , related by a rotation of the SiO₄ tetrahedra around their C₂ axes. The corresponding internal coordinate, the tilt angle δ (ref. 3), is negative in the α_1 phase, positive in α_2 and zero in β .

The net charges calculated by this method should provide reliable trends of ionicity in connected structures when identical basis sets are used. We find that the net charge on silicon decreases

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Unit-cell energy (a.u.) versus tilt angle for cristobalite SiO_2 (dotted line) and GeO_2 (dashed line). The origin of the potential corresponds to the α twinned structure.

from the α to the β structure by 0.018 a.u. in quartz and 0.014 a.u. in cristobalite. In fact, no net changes in ionicity between any of the crystal phases are predicted by our *ab initio* calculations on infinite crystals.

The Born-Oppenheimer energy curve $E(\delta)$ suggests that the high-temperature β phase can be viewed not as a minimum of the potential-energy surface but rather in terms of the expectation values of the atomic coordinates with respect to the nuclear wavefunction. Molecular dynamics calculations⁴ provide support for this interpretation. Starting from the α_1 structure, the α_2 twin appears as the temperature is raised, and at 850 K the system alternates dynamically between

the two phases. This corresponds to the appearance of a symmetric double-well potential in the Born-Oppenheimer energy surface (see figure). The soft-mode normal coordinate can be approximated by the tilt angle δ . For the symmetric double well, the expectation value $\langle \delta \rangle = 0$, corresponding to the β phase, whereas at low temperature the symmetry is broken and $\langle \delta \rangle$ takes negative and positive values for α_1 and α_2 , respectively. When the nuclei are considered as quantum particles, the $\alpha \rightarrow \beta$ transition is displacive, in agreement with experiments⁵, whereas a classical description corresponds to the dynamical picture of the molecular dynamics calculation⁴.

As pointed out by Tsuneyuki *et al.*⁴, the thermal energy required to symmetrize the potential increases with the height of the barrier separating the twinned structures. We calculate a higher barrier for GeO_2 than for SiO_2 (see figure), which explains why no β structure is observed for the germanium analogues of silica polymorphs.

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Calcium binding to fibrillin?

SIR — Dietz *et al.*¹ described an Arg 239→Pro mutation in one of the 34 epidermal growth factor (EGF)-like repeats of the fibrillin gene² found in two patients with Marfan syndrome. These repeats contain a consensus sequence (see diagram) analogous to that found in the first EGF-like domain of coagulation factor IX which is known to bind calcium³. It therefore seems likely that fibrillin will also bind calcium. Moreover, the two-dimensional NMR structure of the EGF-like domain⁴ of factor IX suggests that Arg 239 in the analogous fibrillin EGF-like domain is located on

the second strand of an antiparallel β -sheet adjacent to the inferred calcium binding site. We propose that local disruption of this calcium-binding site could be responsible for the defect in fibrillin function, although long-range effects of the proline substitution on protein structure cannot be excluded. Supporting the concept of local disruption is the identification of two mutations in the factor IX EGF-like domain, Asp 47→Glu and Asp 64→Asn, present in patients with haemophilia B. These mutations reduce calcium binding appreciably but do not grossly affect the protein's structure³.

Several groups have proposed that EGF-like domains are involved in protein-protein interactions. We would like to draw attention to a recent paper⁵

HFIX (I) (47-84)	D	G	D	Q	CESNPCLNGGSK	D	DINSYECWCPFGFEGKNCEL
(II)	D		D	E		N	
(III)	D		D	E		N	
FIBRILLIN (215-256)	D	I	D	E	CQRDPLLCRGGVCH	N	TEGSYRCECPFGHQLSPNISACI
						239 ↓ P	

Amino acid residues important for calcium binding to the human factor IX (HFIX) EGF-like domain (I) are shown boxed with the analogous residues from one EGF-like domain of fibrillin. The position of the mutation Arg 239→Pro identified in two patients with Marfan syndrome is indicated. Variations of the calcium-binding consensus found in other proteins, which also bind calcium when introduced into factor IX are shown (II, III).

where *notch* and *delta*, two *Drosophila* proteins containing EGF-like repeats with the calcium-binding consensus, interact in a calcium-dependent manner. Could calcium interaction with EGF-like repeats in fibrillin be intimately linked with its function in connective tissue?

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Bell's inequality

SIR — Maddox's article¹ on non-locality in quantum mechanics reminded me that Bell's inequality has a long history which may be of some interest. A few years ago, I attempted to trace the origins of the probability metric. (The distance between two events is the probability that one of them occurs but not both. Strictly speaking, this is a pseudo-metric from which a metric can be constructed by standard techniques.) My colleague, D. A. Edwards, pointed out that the treatise by Dunford and Schwartz² contains both a discussion of a more general metric on measure spaces and a survey of its history. There it is traced back to work of Aronszajn and Nikodým³ in the late 1920s. Moreover, that metric is actually just a special case of the L^1 metric on integrable functions discussed 10 years earlier by Fréchet⁴. (One restricts the L^1 metric to indicator functions of events.)

The fact that the basic inequality was known for so long makes it all the more surprising that, until Bell's independent rediscovery of it in the 1960s, no one seems to have observed that the triangle inequality fails in quantum mechanics and can therefore provide a test of large classes of hidden variable theories. The new paper by Fivel⁵, which Maddox discusses, provides an interesting new insight by comparing the probability metric with the quantum-mechanical Hilbert space metric and thereby isolating the mechanism which gives rise to Bell's inequality in one case but not in the other.

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Quantal synaptic transmission?

SIR — Larkman *et al.*¹ suggest that excitatory postsynaptic potentials (e.p.s.ps) in the CA1 region of the hippocampus fluctuate in a quantal manner. This finding may permit detailed analysis of the mechanisms underlying long-term potentiation and other forms of synaptic plasticity. However, it can not be reconciled with the findings of two other studies using a similar preparation which suggest either a continuous synaptic amplitude distribution² or a quantal amplitude nearly an order of magnitude smaller³. I believe that the data of Larkman *et al.*¹ are consistent with non-quantal synaptic fluctuations.

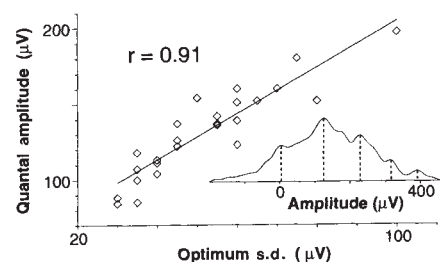
To demonstrate quantal behaviour, Larkman *et al.* present synaptic amplitude histograms with regularly spaced peaks, and analyse these histograms using a conventional technique (maximum likelihood estimator deconvolution, MLED). This technique assumes that the processes generating baseline noise are independent of those generating synaptic amplitude fluctuations, which predicts that the standard deviation of the noise (σ_n) will not be correlated with the quantal amplitude (Q).

When MLED is applied to a simulated amplitude histogram constructed from a continuous (non-quantal) amplitude distribution, or a poorly resolved distribution ($Q < \sigma_n$), it produces a potentially misleading result consisting of discrete components separated by 1.5 to 2.2 σ_n (ref. 4). Under these conditions the optimum σ_n , as used by Larkman *et al.*, will be highly correlated with Q , and a linear regression of Q versus σ_n will have a slope close to 1.5. The values of Q and optimum σ_n for 25 e.p.s.ps presented by Larkman *et al.* in their Table 1 were highly correlated ($r=0.91$) and the linear regression of Q versus σ_n had a slope of 1.5 (see figure). Q and measured σ_n were also correlated ($r=0.68$). These results are consistent with the hypothesis that e.p.s.p. amplitudes vary in a continuous manner, and leads to the question of how the peaks in the amplitude histograms arose. It is possible that they resulted from sampling error because of the relatively small sample size used (median number of trials = 600), combined with the procedure used to define a 'period of stability' and to select an epoch for analysis from the much larger sample of peak amplitudes (up to 12,000).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

To test this possibility, a random sample of 5,000 points was drawn from a gaussian distribution chosen to have the same mean and standard deviation as one of the amplitude histograms of Larkman *et al.* (their Fig. 1d). I selected an epoch of 600 consecutive points from the stimulated amplitude measurements and produced a histogram with clear peaks (see figure). The location of these peaks



Mean quantal amplitude versus optimum standard deviation for data taken from ref. 1, Table 1. The optimally fitted regression line and the correlation coefficient, r , for Q against σ_n are also shown. The inset shows a simulated smoothed amplitude histogram sampled from a continuous gaussian distribution. The peaks, which arose from sampling error, are indicated with vertical dashed lines.

remained constant when the epoch was subdivided.

This result demonstrates that regularly spaced peaks in a synaptic amplitude histogram can arise from sampling error, even when synaptic fluctuations are non-quantal. It is consistent with the observation¹ that clear peaks do not occur when larger sample sizes are used. A good illustration of the tendency of clear peaks to disappear as sample size increased is provided by Larkman *et al.* (their Fig. 3d), where the peak-to-valley ratio progressively and significantly decreased as more samples were added. If true quantal peaks were present in their data, the peak to valley ratio should have remained approximately constant.

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LARKMAN *ET AL.* REPLY — We believe that Clements is mistaken in a number of respects. First, the "continuous synaptic amplitude distribution"² to which he refers was a study of the amplitude distribution of *miniature* synaptic currents, putatively single quanta. Second, our quantal amplitudes (expressed as peak voltage) are not an order of magnitude larger than would be expected from the peak amplitudes of presumed quantal synaptic currents measured by Malinow³ and others².

Third, the procedure we used to analyse amplitude histograms did not re-

quire that the processes generating the signal and contaminating noise were independent. On the contrary, we proposed that a significant component of the noise was due to spontaneous release of quanta from the terminals generating the e.p.s.p. This hypothesis implies that there should be a correlation between quantal amplitude and noise standard deviation (confirmed by us in simulation studies). Such a correlation has already been observed in experiments by Korn *et al.*⁵, where the ratio (Q/σ_n) was greater than three.

Finally, if the MLED procedure is applied to a simulated 'poorly resolved distribution' it is true that a misleading result can be obtained. The average separation of the discrete components will depend on the details of the computer program used and the exact simulation conditions. For example, Sayer and Redman (see ref. 6) found an average of 1.2–1.4 σ_n rather than the 1.5–2.2 reported by Clements⁴. Nevertheless, our results are not seriously affected even if a value of 2.2 σ_n is set as the criterion. The mean separation in our data was 2.61 ± 0.35 (s.d.) times optimum σ_n with 22 of the 25 histograms having a separation above 2.2. It is true that the slope of the relationship in our data is roughly 1.5, but the regression line in Clements' figure does not go through the origin.

For these reasons we do not believe that the points made by Clements affect our interpretations. We do, however, agree that 'peaky' histograms can readily arise from sampling error. We had performed fairly extensive, but not exhaustive, simulations and were satisfied that clear peaks occur more commonly in our data than would be expected by chance. (If the peaks had arisen solely by chance, we would not have expected the peak separation to be so closely correlated with the measured noise s.d.) The changes we observed in mean e.p.s.p. amplitude over time led us to consider that there might be non-stationarity in quantal size (this being a possible factor contributing to the variance in spontaneous synaptic currents observed by others².) If there is any such non-stationarity, the peak-to-valley ratio would not remain constant.

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The Great White Spot and disturbances in Saturn's equatorial atmosphere during 1990

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A giant storm, the Great White Spot, erupted at the end of September 1990 as a localized, bright cloud system close to Saturn's equator. Its evolution produced a complex planetary disturbance which affected the whole equatorial region a month later. Similar spots have appeared approximately once every saturnian year (about 30 Earth years), implying that a seasonal change—in solar heating, for example—may be responsible for their occurrence.

The observation at ground-based resolution of isolated features in Saturn's atmosphere is a difficult task. In contrast to Jupiter, where a variety of cloud forms are observed even with very small telescopes, Saturn hardly shows its characteristic alternating pattern of belts (low albedo bands) and zones (high albedo bands) arranged parallel to the equator¹⁻³. The presence of an optically dense haze in the upper cloud layers seems to be the reason for Saturn's bland appearance⁴.

Until 1990, ground-based telescopes had tracked only about 30 spots with enough accuracy to allow the differential rotation of Saturn's upper atmosphere to be inferred². The high-resolution images obtained during the Voyager encounters with Saturn in 1980–81 provided a detailed view of cloud morphologies and of the dynamics of the visible atmosphere⁵⁻⁷. Among the spots reported in ground-based data, some are conspicuous and remarkable because of their high activity and high spatial scales. We call these phenomena 'Great White Spots' (GWS) by analogy with the jovian Great Red Spot^{2,8}. Before 1990, such an event was recorded on only four occasions and at very different latitudes: 1876.9 (saturnigraphic latitude $\phi_g = 5^\circ$ N); 1903.5 ($\phi_g = 36^\circ$ N); 1933.7 ($\phi_g = 5^\circ$ N); 1960.25 ($\phi_g = 58^\circ$ N) (refs 2, 8). The spots appeared at a frequency similar to the planet's orbital period around the Sun, coinciding with the saturnian mid-summer season in the northern hemisphere. In view of this recurrence, we suggested⁹ that a GWS might appear around 1990. The fifth event was discovered¹⁰ near to the equator at the end of September 1990. Here we describe our telescopic imaging and a first analysis of this GWS and its related disturbances.

Observations

Our coordinated work to study Saturn's northern hemisphere extends back to 1979 (ref. 11). For the 1990 apparition, our survey started in June at Pic-du-Midi Observatory. A series of images was obtained with the 1.05-m telescope and a Thomson CCD camera equipped with broadband and interference filters.

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TABLE 1 Filters used and spectral ranges covered

Nomenclature	λ_{eff} (Å)	$\Delta\lambda$	Detector	Comments
Vio	3,850	1,000	TP 2415	—
B	4,800	800	CCD	—
Vis	5,000	—	TP 2415	—
V	5,900	1,400	CCD	Includes 6,190 CH ₄
R	6,500	1,500	CCD	Includes 6,190 and 7,250 CH ₄
I	8,600	2,000	CCD	Includes 8,990 CH ₄
RG-695	8,100	—	CCD	Includes 7,250 and 8,990 CH ₄
CH ₄ 6,190	6,184	19	CCD	Isolates 6,190 CH ₄
6,000	6,046	40	CCD	Adjacent continuum
CH ₄ 7,250	7,251	18	CCD	Isolates 7,250 CH ₄
7,500	7,508	50	CCD	Adjacent continuum
8,300	8,291	46	CCD	Adjacent continuum
CH ₄ 8,930	8,932	45	CCD	Isolates 8,990 CH ₄

During the evolution of the GWS, the Pic data covered the period from 27 September to 21 December 1990. These images were processed in two different ways, one to retrieve photometric data, and the other to enhance contrast for morphology studies and for zonal wind measurements. To support these last investigations, a series of photographs covering the period from 1 October to 13 November was taken at visible and violet wavelengths with two 40-cm telescopes. Table 1 shows our wavelength coverage, evaluated after combining the filter transmission curves with the detector response (CCD or photographic film). In total, we imaged Saturn on 48 different nights during the whole interval.

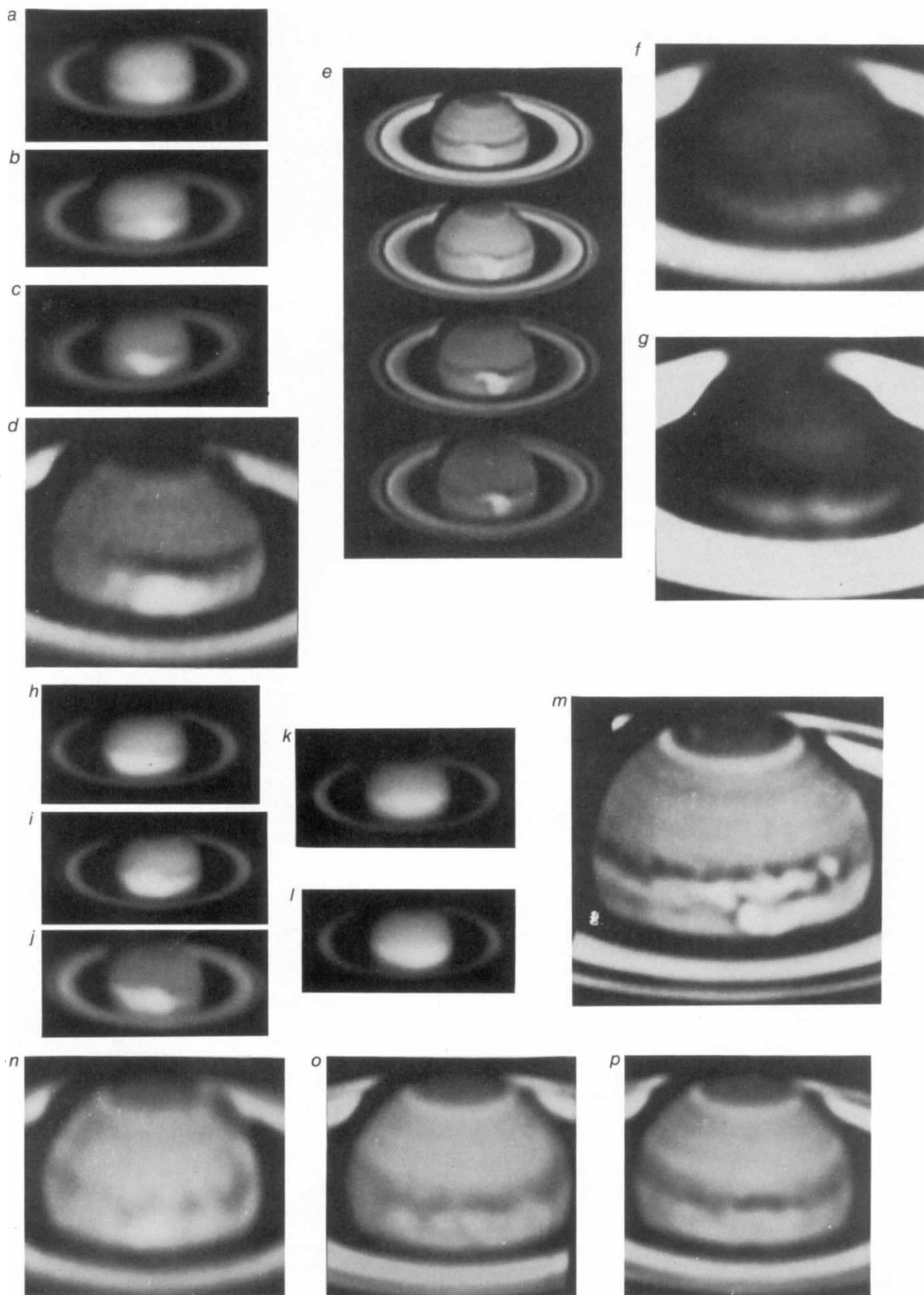
Throughout this work, features in Saturn's disk are located by their saturnigraphic latitudes and by systems I and III reference frames for longitudes. System I has a rotation period of 10 h 14 min (844.3° per day) and is appropriate to identify the spots moving with the strong equatorial jet, whereas system III (assumed to be the rotation of the planet's interior) has a period of 10 h 39 min 24 s (810.76° per day) and is used as a reference for wind velocities.

Morphology and evolution

Before the outbreak of activity, the two permanent bands of Saturn's equatorial atmosphere were very distinct. The equatorial zone (EZ), bright in the visible and near infrared but dark in violet and ultraviolet, extended from the ring projection southward of the equator to the latitude 13° N. Next to it was the north equatorial belt (NEB), darker in the visible than in the ultraviolet, located between 13° N and 25° N. These positions have remained practically unchanged during the past decade^{11,12}.

Because of the different structures and stages of development observed in the GWS, we have divided the morphological analysis into four separate phases.

First phase (25 September to 5 October; Fig. 1a–g). The Great White Spot was discovered as a conspicuous bright structure on 25 September¹⁰. It was initially located at $\phi_g = 12^\circ \pm 1^\circ$ N in the interface between the EZ and the NEB, and at longitude



309° (system I). Over the following days the spot grew in size and brightness. Our first photographs, taken on 1 October, revealed the spot as a tilted ellipse, with slightly diffuse edges, with its long axis at $\sim 47^\circ$ relative to the latitude circle (Fig. 1a). Its main body appeared homogeneous and had dimensions of 18,600 km (east–west) by 26,300 km (north–south). If the diffuse boundaries were included, the spot extended up to $\sim 30,000$ km. On 2 October (21 hours later), we detected a counterdirectional zonal expansion starting in two sites, one in the spot's north-western extremity at latitude $16^\circ \pm 2.5^\circ$ N (we call this GWSf) and the other lying to the southeast at latitude $7^\circ \pm 1^\circ$ N (GWSp) (Fig. 1b, e). The flow in GWSp was eastward along the southern edge of the NEB, whereas that in GWSf was westward along the central portion of the NEB. Both of these flows were relative to the spot's geometric centroid which was then located near 9° N. This motion was produced by the strong zonal wind shear between 5° N and 20° N. At the same time we noted an increase of the spot's main body. Although it was difficult to separate both motions, we were able to make time-lapsed area measurements of the GWS nucleus. If the area increase is the result of mass outflow from the source at upper levels, then we can estimate the divergence of the wind in the outflow layer¹³, which from 1 to 4 October was $\text{div}_h \mathbf{v} = (1/A)(dA/dt) \approx 3 \times 10^{-6} \text{ s}^{-1}$. Here A is the area and $\mathbf{v}$ the wind speed. This value is of the same order as that measured in previous events⁸.

Meanwhile, the spot continued to expand (Fig. 1c), and by 5 October the initial source had developed a double nucleus (features WS1 and WS2) which was very distinct in our near-infrared and methane band images (Fig. 1d, f, g). By this time the disturbance was $\sim 95,000$ km in extent, and its brightness had started to decrease. Moreover, the NEB was very dark at longitudes close to the GWS (Fig. 1d). Feature WS2 seems to correspond to the original storm source. It seems, however, that during the growing phase the spot's centroid, as seen in the red, moved from $\sim 12^\circ$ N (1 October) to $\sim 5^\circ$ N (5 October and afterwards). This is also suggested by the apparent change in zonal velocity of the storm nucleus from $u \approx 365 \text{ m s}^{-1}$ (25 September to 4 October) to $u \approx 402 \text{ m s}^{-1}$ (5 October to 18 November).

Second phase (6 October to 31 October; Fig. 1h–l). During this period the two front-like cloud bands (GWSp and GWSf) moved rapidly in opposite directions relative to the GWS centre (Fig. 1h–i). The high-albedo clouds covered practically the whole NEB (which was then a bright band) and extended partly into the northern half of the EZ (Fig. 1k–l). Some bright 'knots' were observed superimposed upon the general brightening of white clouds overlying the NEB. Around 21 October, both fronts merged at longitude 240° (system I). The whole area between latitudes 10° N and 22° N was covered by these clouds. The 'double spot' (WS1 and WS2) was bright and distinct and was partly detached from this band (Fig. 1j). Both features were nearly stationary in system I and, despite some changes in their shape, they survived during November. This storm centre is shown in images obtained with the Hubble Space Telescope on 9 November¹⁴.

Third phase (November; Fig. 1m, n). A new isolated bright structure (WS3) was discovered on 2 November near longitude 150° (system I). It was located south of the first event, close to the equator at $\phi_g = 2^\circ$ S, and extended 11,600 km E–W and 13,700 km N–S. Our red and near-infrared images of 5 November, taken under excellent seeing conditions, revealed a series of low- and high-albedo spots located at different latitudes north of WS3 (Fig. 1m). At 18° N, dark spots, each $\sim 7,500$ km in diameter, appeared at regular intervals of $\sim 13,000$ km (which is roughly the size of WS3). These could correspond to the space left by the undulating strip of white clouds observed in the Hubble Space Telescope images. South of these spots and east of WS3, a pattern of bright spots of dimensions $\sim 10,500$ km (E–W) and $7,000$ km (N–S) was observed at 10.5° N. At 5.5° N, a group of dark spots were inserted along the southern edge of these white spots, their shape being oval with dimensions of $10,000$ km (E–W) and $6,500$ km (N–S). A wavy thin dark belt centred at the equator was observed west of WS3.

During the first three weeks of November, new bright, transient features appeared and evolved in the EZ and NEB. The latter belt acquired a complex texture, with light and dark patches encroaching upon it (Fig. 1n) and extending up to the parallel 30° N.

Fourth phase (1 to 21 December; Fig. 1o–p). After early December, the NEB became dark and more uniform, especially in its northern half. In the southern half near 12° N, however, there were rapidly changing dark patches and elongated streaks. A series of equally spaced bright spots was framed between the NEB and EZ at 8° N (Fig. 1o). Their diameters were $\sim 10,000$ km, and they were surrounded by dark material along their north and south edges. The texture of this active region was similar to that usually observed in the jovian NEB.

Less active clear areas evolved along the southern half of the EZ. By mid-December, with Saturn nearing conjunction with the Sun, we observed a larger prominent NEB (meridional amplitude $\sim 16^\circ$), its southern edge being displaced toward the equator. Similar behaviour, with strong changes in the NEB following the outbreak of bright spots, was also reported^{3,8} during the events of 1903 and 1933.

Zonal winds

After identifying the features on successive images, separated by a minimum of three days, we converted the measured x – y projected coordinates (in a tilted oblate planet) into longitude and latitude. We assumed that the cloud systems acted as tracers of Saturn's atmospheric winds. Least-square fitting of their longitude against time allowed us to evaluate their rotation period and zonal velocity relative to system III. In total, we tracked 25 individual features (size $> 7,000$ km) imaged at red and near-infrared wavelengths (filters Vis, R, I and RG-695). There are two main sources of error in our zonal wind measurements. One is related to the location of Saturn's disk edges and the centroid of feature, and depends strongly on the observing conditions (seeing and number of suitable images). We estimate a r.m.s. error ~ 1 – 3° in our longitude and latitude determinations. The other is related to the number of independent points available in the longitude–time diagram for each feature, and to the extent of its temporal coverage. From the least-square fit we get uncertainties of ~ 5 – 15 ms^{-1} in our zonal wind velocities.

In Fig. 2, the velocity of the equatorial zonal wind measured during 1990 between latitudes 18° N and 8° S is plotted against planetographic latitude. These values are superimposed on the velocity profile obtained by the Voyager team in 1980–81 (ref. 7), and on other values derived from historical ground-based data spanning a considerable time interval^{2,8}.

Our observations extend the previously known profile to the southern part of the EZ, a region unexplored by the Voyagers. Note that there is good overlap between the end of the Voyager profile at 2° S, our data from 2° N to 8° S and the spot observed² at 12° S. The correlation between all these data supports the

FIG. 1 Images of the development of Saturn's GWS and equatorial disturbances in 1990 (date, ur, filter, central meridian Systems I and III): a (1 Oct, 12 h 54 min, Vis, 339.7 I, 328.6 III); b (2 Oct, 9 h 39 min, Vis, 349.6 I, 309.5 III); c (4 Oct, 2 h 21 min, Vis, 340.6 I, 244.3 III); d (5 Oct, 19 h 31 min, infrared (I), 349 I, 194.9, III); e (2 Oct, from top to bottom: 19 h 38 min, infrared (I), 314.1 I, 286.7 III; 19 h 43 min, red (R), 316 I, 289.5 III; 19 h 48.5 min, yellow (V), 319.9 I, 292.3 III; 19 h 55.5 min, blue (B), 324 I, 296.2 III); f (5 Oct, 20 h 20 min, CH_4 , 7,250 Å, 17.4 I, 222.5 III); g (5 Oct, 19 h 46 min, CH_4 , 8,990 Å, 357.5 I, 203.3 III); h (10 Oct, 11 h 23 min, Vis, 324.1 I, 13.6 III); i (13 Oct, 11 h 02 min, Vis, 324.3 I, 273.8 III); j (13 Oct, 11 h 09 min, Vis, 328.4 I, 276.6 III); k (17 Oct, 10 h 50 min, Vis, 94 I, 269.8 III); l (19 Oct, 11 h 18 min, Vis, 358.8 I, 17.1 III); m (5 Nov, 17 h 24 min, RG-695, 164 I, 54.2 III); n (18 Nov, 18 h 01 min, OG-590, 360 I, 174 III); o (2 Dec, 16 h 47 min, I, 254 I, 320.7 III); p (7 Dec, 17 h 05 min, RG-695, 165 I, 64.5 III). North is up in all these images.

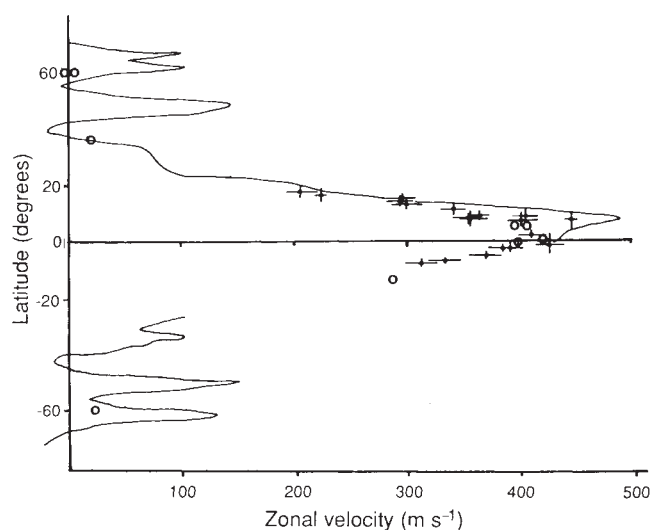


FIG. 2 Zonal wind velocity in Saturn's atmosphere: (+), measurements in 1990 of cloud systems related to the equatorial disturbance (this work); (—), data from Voyager team obtained in 1980–81 (from ref. 7); (○), selection of outstanding features from historical records (refs 2, 8).

idea that the zonal wind profile persists over long periods, although some changes are apparent. Our data indicate a slight asymmetry of the equatorial jet, with a large meridional shear in its southern part.

It seems that the features we used to measure the zonal wind are distributed at different heights. This is indicated by their different reflectivity when different methane band filters are used, as well as by their latitude. Ground-based and Voyager photometric observations of Saturn's equatorial region indicate that, typically, cloud tops in the EZ and NEB are separated by ~ 150 mbar (~ 30 km) or 0.8 times the gas scale height⁴. Moreover, thermal wind measurements made during the Voyager epoch showed that vertical wind shears of ~ 20 m s⁻¹ per scale height are expected at these latitudes¹⁵. From these considerations, we expected to see a signature of the height dependence of the zonal wind in our data. Although it could be masked by the uncertainty in our measurements, we in fact see no clear evidence of the vertical wind shear. This could indicate that the feature's cloud tops are confined to within one scale height or even less.

Photometry and vertical structure

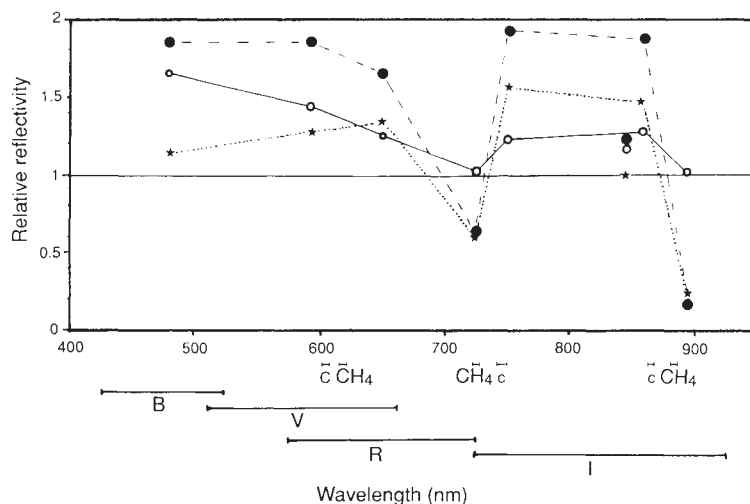
We have determined the spectral dependence of the relative reflectivity of the initial GWS from high-quality photometric images taken on 5 October. To improve the signal-to-noise ratio, series of images were flat-field corrected, recentred, scaled and summed in groups of 4–9 for each filter. We then measured the mean reflectivity on a circle of ~ 2 arcsec² around GWS

maximum. In all these measurements the spot was near the central meridian ($\leq 30^\circ$). The reflectivity was measured relative to the maximum of the rings' ansae, which are the ends of the rings of Saturn, as seen from Earth (an east and west mean). We have also compared it with the reflectivity of an area at the same latitude and in the central meridian of the EZ, on images obtained next day (6 October) in a region not disturbed by the GWS and located $\sim 100^\circ$ of longitude from it.

Our results for the relative spectral reflectivities in various wavelengths between the GWS (max) and the EZ are shown in Fig. 3. The numerical values in some of the filters used (listed in Table 1) are: broadband filters (error ~ 0.01), $R_B = 1.64$, $R_V = 1.43$, $R_R = 1.24$ and $R_I = 1.18$; methane*/continuum filters, $R_{7,250}^* = 1.02 \pm 0.03$, $R_{7,500}^* = 1.23 \pm 0.01$, $R_{8,300}^* = 1.27 \pm 0.01$ and $R_{8,930}^* = 0.97 \pm 0.1$, where the subscript denotes wavelength in angstroms. The GWS had its maximum contrast in the blue, showing at all wavelengths a higher brightness than its surroundings. In the strong methane bands, the reflectivity is very similar to that of the EZ, although the errors in this result are greater, because of the strong ring contamination at these wavelengths and longer exposure times (blurring the image). We note, however, that the contrast of the spot was high and its edges well delineated under the methane filters (Fig. 1f, g).

A comparison with our photographic photometry analysis of the 1933 GWS (ref. 8) showed this event to have a fairly constant relative brightness (relative to its surroundings) of 1.25 ± 0.05 from $\sim 3,800$ to $6,500$ Å. Thus the 1990 event appears to have been much brighter at blue-yellow wavelengths than the 1933

FIG. 3 Reflectivity of the GWS on 5 October 1990 relative to that of the EZ central meridian 100° away, on 6 October (○, —). For comparison we also show the reflectivity of the GWS (●, ---) and that of the EZ (★, ···), both relative to the ansae of the rings. The wavelength coverage of the broadband, methane (CH₄) and adjacent continuum (c) filters is also indicated.



event. Another interesting observation was that the initial spot remained bright in violet and blue light when close to the limb, as is also shown in some images obtained in October at the European Southern Observatory (refs 14, 16, and personal communication from the European Southern Laboratory).

We interpret these results in terms of current models of Saturn's vertical upper clouds and aerosol distribution in the EZ^{4,17}. The high albedo of the GWS in the continuum is a signature of optically thick clouds, and the dependence of the reflectivity on wavelength, in particular the high violet-blue brightness, may be explained by an increase in the continuum single-scattering albedo of GWS cloud tops relative to that of the neighbouring EZ. This suggests a possible reduction of the colouring agent (tropospheric absorber aerosol) responsible for the visible continuum spectrum of Saturn⁴. We propose that this could occur when ammonia, or even water, transported by vigorous ascending motions (see below) forms a mantle of ice crystals on the aerosol particles, which serve as condensation nuclei. On the other hand, the GWS visibility in violet when near the limb, as well as its similar reflectivity to that of the EZ in the strong methane bands, indicates that the storm cloud tops are high, being at the same level or somewhat higher than the EZ cloud tops (optical depth ~ 1 at pressure $P \approx 120$ –240 mbar, as sensed by our 8,930-Å CH₄ filter; see also Fig. 16 in ref. 4).

We would like to emphasize that this analysis is valid only for the initial GWS (2–5 October). Photometry performed on bright spots observed in mid-November showed a lower red reflectivity, with the spots being invisible under the 7,250 and 8,930-Å filters. We believe this could arise because these spots were at lower altitudes.

Discussion of the dynamics

The eruptive nature, rapid growth, relatively high altitude and optically dense cloud tops of the initial GWS outbreak suggest that the storm had a convective origin. Sanchez-Lavega and Battaner⁸ have concluded that, with reasonable values of the parameters for Saturn's atmosphere involved in this one-dimensional model, moist convection originating in the water clouds ($P \approx 10$ –12 bar) could propel parcels of gas up to the troposphere ($P \approx 100$ –250 mbar), where ammonia or water condenses to form the storm cloud tops.

The rarity of the GWS phenomenon suggests the action of rather special conditions in Saturn's atmosphere. It is noteworthy that the disturbance seems to be independent of the

latitude (from 5° N to 58° N), zonal wind magnitude (with velocity extremes of -4 m s^{-1} to 450 m s^{-1}) and probably of the meridional wind shear. But the most outstanding observation is the apparent recurrence of the storm roughly every 30 years (nearly coinciding with Saturn's orbital period of 29.45 Earth years) and near to the summer solstice in the planet's northern hemisphere⁸. This indicates that a seasonal effect must take part in the outbreak of activity. Care must be taken, however, in assigning a unique periodicity to such phenomena, because the observations are biased by the blocking (total or partial) of half of the globe by Saturn's rings, alternating every ~ 13 –15 yr. Calculations^{18,19} of the solar flux incident on the top of the atmosphere show that GWS outbursts took place during the epochs of prolonged intense insolation in the latitude of interest⁸. The outbursts have emerged ~ 12 –16 yr after a deep minimum in the daily insolation rate; to sum up, the time delay relative to this minimum for each event was: 15.7 yr (GWS 1876.9; 5° N); 15.8 yr (GWS 1903.5; 36° N); 13.6 yr (GWS 1933.7; 5° N); 13.7 yr (GWS 1960.25; 58° N); 11.9 yr (GWS 1990.9; 12° N). We propose that a change, of seasonal origin, in the vertical temperature gradient of the upper troposphere ($P < 0.6$ bar) may favour the vertical ascent of thermals that constitute the GWS nucleus. Note that on Saturn the radiative time constant is long and depends strongly on height, producing an altitude-dependent phase lag relative to sunlight forcing of ~ 5 –7 yr in the levels of interest^{20,21}.

The next stage of development after the outbreak was characterized by the zonal and meridional expansion of the disturbance (confined between latitudes $\sim 8^\circ$ S and 30° N) in the environment of high cyclonic wind shear. Some of the periodically spaced features and undulating structures, described earlier, could be associated with a wave phenomenon. A wavelength of roughly the size of the convective centre ($\sim 13,000$ km) is suggested by the regular separation of the features; the wave can therefore be excited by it. The weakening of the GWS involved the cessation of the convective activity and the dispersion of cloud elements by the meridional wind shear. These effects are probably accompanied by sublimation of the ice particles in the clouds by strong insolation, and by their fall by turbulent diffusion.

Finally, we would like to mention that our observations from February to July 1991 (after the Sun-Saturn conjunction) showed some signatures of activity with small, isolated, low-contrast spots along the central part and southern edge of the broad NEB. □

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Mutations affecting body organization in the *Arabidopsis* embryo

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A systematic search for mutations in the flowering plant *Arabidopsis thaliana* that disrupt the spatial organization of the seedling by altering embryogenesis is described. Mutations in nine genes affect three different aspects of the body organization: apical-basal pattern along the single axis of polarity, radial pattern involving the primary tissues, and shape. The results suggest principles of pattern formation in the plant embryo.

PATTERN formation during embryogenesis has been studied intensively in animals by a variety of means including experimental interference, mutational dissection, and molecular analysis¹. In one animal model system, *Drosophila melanogaster*, where all these approaches can be applied, the analysis has led to a fairly detailed understanding of how the basic body pattern is laid down in the embryo (for review, see refs 2, 3). By contrast, relatively little is known about the way the body organization is established in the embryo of higher plants.

We have taken a genetic approach to dissect the process of pattern formation in the *Arabidopsis* embryo. *Arabidopsis* is especially suited for this kind of analysis because of its small size, short generation time, small and truly diploid genome, and the ease of whole-mount analysis of developing embryos (for review, see refs 4, 5). These features enable saturation mutagenesis experiments aimed at identifying genes that are involved in pattern formation. Here, we describe the types of mutants found in our systematic search and discuss their significance in terms of principles of pattern formation in the plant embryo. We begin by briefly introducing the body organization of the seedling, as this is the developmental stage at which the basic body pattern is expressed in higher plants.

Body organization of the seedling

Flowering plants form a taxonomic group of closely related species⁶. Although their adult forms may differ considerably, flowering plants pass through very similar stages of embryogenesis producing the seedling, a juvenile plant of highly stereotyped appearance^{7,8}. The seedling has a simple body organization, which may be described as the superimposition of two patterns, one axial and one radial (Fig. 1). The apical-basal pattern is arranged along the single axis of polarity. From top to bottom, the following elements can be distinguished: shoot meristem, cotyledons, hypocotyl and root including the root meristem. The two meristems give rise to the adult structures during postembryonic development. The radial pattern which is most clearly seen in the hypocotyl of the seedling, involves the three major tissues of the primary plant body: the outer epidermis, the inner mass of ground tissue, and the centrally located vascular strands. Each of the apical-basal pattern elements consists of, or gives rise to, all three tissues.

The body organization of the seedling must originate during embryogenesis although it is not known when or how this occurs.

Descriptive embryology has accumulated much information about morphological and histological changes during embryogenesis, which indicates that some aspects of spatial organization become apparent in the early embryo (Fig. 1; for review, see ref. 7). For example, the primordia of the primary body tissues appear successively in the globular-stage embryo, and other primordia of seedling structures become recognizable at the heart-shape stage (for details see legend to Fig. 1). Subsequent embryogenesis predominantly involves further growth of the primordia by cell division and the differentiation of cells. Thus, morphological evidence suggests that the basic body pattern has been laid down by the heart stage.

In our screen, we preselected putative pattern mutants on the basis of altered body organization of the seedling⁹. The rationale for choosing the seedling stage, as opposed to an earlier developmental stage, was to eliminate mutants defective in more general cell functions, most of which would lead to embryonic lethality (discussed in ref. 9). Mutants with conspicuous seedling phenotypes were subjected to complementation analysis, which defined nine genes each represented by eight mutant alleles on average (Table 1). Phenotypic studies of mutant seedlings and early embryos indicate that these mutations affect three different aspects of the body organization: apical-basal pattern, radial pattern and shape.

Apical-basal pattern deletions

Mutations in four genes delete large regions of the apical-basal pattern without affecting the formation of the primary body tissues. On the basis of the relative positions of defect observed in the strongest mutant phenotypes, we distinguish four main classes of pattern deletions: apical, basal, central and terminal (Fig. 2). Two complementary pairs of apical and basal as well as central and terminal deletions add up to the complete apical-basal pattern, suggesting that the deletion phenotypes define boundaries along the axis. In the following presentation, the mutant phenotypes will be described in morphological terms for sake of simplicity although the pattern deletions need not necessarily coincide with morphological units of the seedling.

In the class of apical deletion, mutant alleles of the *gurke* gene affect the cotyledons and the shoot meristem. These structures are missing in the strongest phenotype (Figs 2 and 3b). The internal phenotype matches the external appearance in that the vascular strands terminate apically without any indication of branching (Fig. 4b). This is unlike the situation found in the apical region of wild-type seedlings where branches of vascular strands extend into the cotyledons (Fig. 4a). Thus, the strongest *gurke* phenotype probably corresponds to the lack of the apical region from the upper end of the hypocotyl. Weaker *gurke* alleles show small apical bulges with short branches of vascular strands, which may represent strongly reduced cotyledonary tissue (data not shown).

Another class of pattern deletion, which affects the centrally located hypocotyl, is represented by mutant alleles of the *fackel* gene (Fig. 2). The cotyledons appear directly attached to the root (Fig. 3c). Our interpretation of the mutant phenotype is supported by the internal structure of mutant seedlings. In wild-type seedlings, the bundle of vascular strands splits just below the level where the cotyledons are inserted into the axis.

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TABLE 1 Mutations affecting body organization in the *Arabidopsis* embryo

Mutant phenotype	Gene (symbol)	Number of alleles
Apical-basal pattern deletion		
Apical	<i>gurke</i> (<i>gk</i>)	9
Central	<i>fackel</i> (<i>fk</i>)	5
Basal	<i>monopteros</i> (<i>mp</i>)	11
Terminal	<i>gnom</i> (<i>gn</i>)	15
Radial-pattern defect		
	<i>keule</i> (<i>keu</i>)	9
	<i>knolle</i> (<i>kn</i>)	2
Shape change		
	<i>fass</i> (<i>fs</i>)	12
	<i>knopf</i> (<i>knp</i>)	6
	<i>mickey</i> (<i>mic</i>)	8

Classification of mutant phenotypes was based on the analysis of seedling and early embryonic phenotypes. Seeds of the Landsberg erecta (Ler) ecotype were mutagenized with 0.3% ethyl methanesulphonate (EMS) for 8 h. Before mutagenesis, the seeds were imbibed for 4 days at 4 °C to break dormancy and then redried for 1 day at 25 °C to arrest development. After mutagenesis,

In *fackel* seedlings, however, the vascular strands separate at the upper end of the root and diverge directly into the cotyledons (Fig. 4c). Another aspect of the *fackel* phenotype involves the cotyledons which are abnormally shaped, and there are also often more than two cotyledons. The apical abnormality may be caused by the absence of a central portion of the axis.

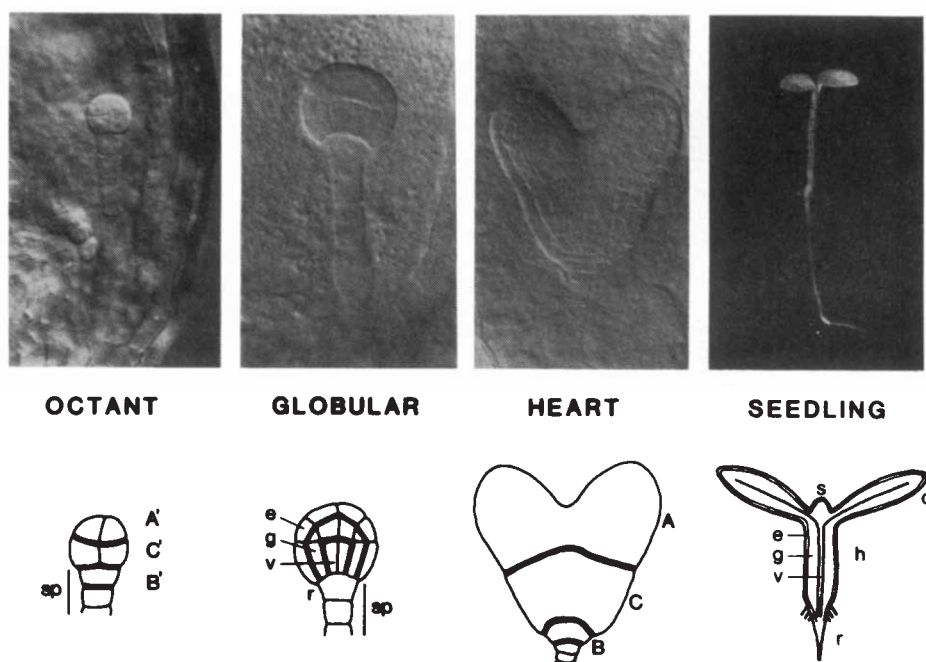
In the class of basal deletion, *monopteros* seedlings show a mutant phenotype that is nearly complementary to that of *gurke* seedlings (Fig. 2). Mutant alleles of the *monopteros* gene eliminate both the hypocotyl and the root. Thus, the mutant seedling essentially consists of an apical piece of axis, including the cotyledons and the shoot meristem, which tapers off basally (Figs 2 and 3d). The number of cotyledons varies from two to one, with intermediate forms showing different extents of fusion. A variable proportion of the mutant seedlings have strongly fused cotyledons or a single cotyledon, and also lack a shoot meristem. The internal structure of the mutant seedling matches its external appearance in that the tissues of the two cotyledons

the seeds were rinsed thoroughly with water and sown in flats containing a soil mixture of potting soil and sand (1:1). The plants were grown at 25 °C under constant illumination. One or two siliques were collected from each plant, and their seeds (families) were plated on 0.3% agar in square Petri-dishes, each with 36 seed families containing F₁ embryos. The agar plates were incubated at 4 °C for 4 days and then exposed to light at 25 °C for another 4 days before the seedling phenotypes were scored. A total of 44,000 independently derived seed families corresponding to 25,000 embryonic-lethal mutations were screened. About 5,000 seed families segregated for seedling phenotypes; from each family up to 10 normal seedlings were transferred to the soil and retested for the segregation of abnormal seedlings in the F₂ generation, which yielded 250 putative pattern mutants. For complementation analysis, heterozygous plants were first identified in segregating lines by selfing. Plants to be used as females in crosses were manually emasculated, 3 or 4 flowers at a time, and cross-pollinated the following day. Noncomplementing mutants were identified by the occurrence of mutant seedlings derived from the crosses. One allele each of the genes *gurke*, *fackel* and *knolle* were isolated on the basis of their mutant seedling phenotypes in an X-ray mutagenesis experiment, in which wild-type (Ler) pollen was irradiated with 20,000 R. All mutants described here are nuclear recessive in that they segregate roughly 25% mutant seedlings on selfing.

are well differentiated, including the formation of vascular strands, although the vascular system is not properly reticulated (Fig. 4d).

The terminal class of pattern deletion affects structures located at the two opposite poles of the seedling (Fig. 2). Mutant alleles of the *gnom* gene delete the root and strongly reduce or eliminate the cotyledons, resulting in seedlings that are cone- or ball-shaped, respectively (Fig. 3e). This phenotypic variability is observed among seedlings carrying the same mutant allele. One extreme case approaches the shape of a ball with no apparent axial organization (Figs 2 and 3e). The external phenotype of mutant *gnom* seedlings is consistent with their internal defects, including the organization of vascular tissue. Although vascular cells are present and well differentiated they are not interconnected to form strands. Ball-shaped *gnom* seedlings that appear to lack apical-basal polarity do display the radial pattern of the three tissues very clearly: underneath the surface layer of epidermis, a sphere of ground tissue surrounds a central 'cloud' of vascular cells (Fig. 4e).

FIG. 1 Embryonic origin of the seedling pattern. The upper panel shows photographs of embryos at the octant, globular and heart stages (whole-mount preparations, Nomarski optics), and a seedling. The lower panel consists of schematic drawings showing relevant features of corresponding stages of development. The body organization of the seedling (4 days after germination) involves two patterns, one axial and one radial. Along the apical-basal axis of polarity, the following elements can be distinguished: shoot meristem (s), cotyledons (c), hypocotyl (h) and root including the root meristem (r). The radial pattern, which is most clearly seen in the hypocotyl, consists of the three primary body tissues: the outer epidermis (e), the ground tissue (g) and the vascular strands (v). The primordia of seedling structures are morphologically recognizable in the heart-stage embryo (30% of embryogenesis, which takes about 14 days from the zygote to the mature embryo). The regions A, C and B marked by heavy lines seem to give rise to the apical-basal pattern elements: A to the shoot meristem and the cotyledons, C to the hypocotyl, and B to the root including the root meristem. The primordia of the radial pattern elements, marked by heavy lines, appear successively in the globular-stage embryo (15–20% of embryogenesis). Regions A, C and B of the heart-stage embryo may be traced back to the upper (A') and lower (C') tiers in the octant-stage embryo (10% of embryogenesis) and to the uppermost cell (B') of its suspensor (sp). The upper daughter cell of B' becomes the founder cell of the root primordium (r) in the globular-stage embryo (see ref. 7).



(B') of its suspensor (sp). The upper daughter cell of B' becomes the founder cell of the root primordium (r) in the globular-stage embryo (see ref. 7).

Embryonic phenotypes of pattern mutants

The pattern mutants were originally selected on the basis of their seedling phenotypes. As mentioned above, the primordia of seedling structures become morphologically recognizable in the heart-stage embryo (Fig. 1). We therefore wanted to determine whether we could distinguish wild-type and mutant embryos at that early stage and if so, whether there was a topographical correlation between the defect in the embryo and the missing pattern element of the seedling.

By comparing heart-stage embryos of wild type and mutants, we could readily distinguish the mutant embryos on the basis of their shapes and/or the absence of specific primordia as judged by morphological criteria (Fig. 5a-e). In *gurke* embryos, the apical surface is smoothly rounded because of the absence of the bulging cotyledon primordia, whereas the rest looks normal. Early *fackel* embryos are broader than normal, which may result from the juxtaposition of root and cotyledon primordia. This early phenotype could also account for the variable number of cotyledons at the seedling stage. Mutant *gnom* and *monopteros* embryos lack the very characteristic arrangement of cells normally representing the root primordium. The two embryonic phenotypes differ in overall appearance: *gnom* embryos look round whereas *monopteros* embryos are triangle-shaped, with the narrow end towards the basal pole. Thus there is generally a topographical correlation between the early embryonic and the seedling phenotypes in apical-basal pattern deletion mutants. This observation strongly suggests that apical-basal pattern formation occurs before the heart stage and that this process critically depends on the actions of the four genes identified.

Radial pattern defects

Mutations in two genes, *knolle* and *keule*, affect the radial pattern which involves the three major tissues: epidermis, ground tissue and vascular tissue. These mutations also change the overall appearance of the seedling so that the analysis of external

features does not reveal their specific effects. The interpretation of the mutant phenotypes was greatly helped by the inspection of internal structures and especially by the study of mutant embryos at the globular stage when the primordia of the radial pattern elements become morphologically apparent in wild-type embryos.

Most *knolle* seedlings look round or tuber-shaped (Fig. 3f). The surface appears rough because of the lack of a well formed layer of epidermal cells. Internally, abnormally large cells enclose lumps of vascular tissue (Fig. 4f). The mutant phenotype can be traced back to the early globular stage when in the wild-type embryo, an outer layer of eight epidermal precursor cells surrounds an inner group of eight cells (Fig. 5h). In *knolle* embryos, one cannot morphologically distinguish an outer layer of epidermal precursor cells from an inner cell group (Fig. 5i). Instead, the early embryo seems to consist of enlarged cells which are irregularly spaced. In addition, the upper end of the suspensor is enlarged as if this region had become part of the embryo.

Mutations in another gene, *keule*, cause defects in the epidermis, which also results in a rough surface. The seedling shape varies, often approaching an elongated structure topped with strongly reduced cotyledons (Fig. 3g). Inspection of internal structures shows that the epidermal cells are bloated and irregularly arranged whereas the cells of both the ground tissue and the vascular strands look normal (Fig. 4g). Thus, the defect seems to be confined to the outer tissue layer. Essentially the same phenotype is observed in the mutant globular-stage embryo (Fig. 5j). The precursor cell of the root primordium also appears larger than normal. Although the epidermal precursor cells form in *keule* embryos, they are abnormal from the very beginning. Thus this gene may be specifically required for the correct development, rather than the initial formation, of a radial-pattern element.

Shape changes

Pattern mutants affect specific elements of the seedling pattern. By contrast, shape mutants are defined here by grossly abnormal seedlings which, however, retain all the pattern elements found in wild-type seedlings. Mutant alleles of the *fass* gene illustrate this point. The mutant seedlings are stout, short, and compressed in the apical-basal axis (Fig. 3h). Yet all pattern elements are present, including root, hypocotyl and cotyledons, although they are abnormally shaped. The number of cotyledons may be increased to three or four, presumably resulting from the enlarged diameter of the seedling. The mutant phenotype is also expressed at the cellular level in that the cells are round and irregularly spaced (Fig. 4h). By contrast, the cells of the wild-type seedling are elongated and stacked. The *fass* mutant embryos can be recognized by the abnormal shape and arrangement of their cells from an early stage (Fig. 5f). As *fass* seedlings contain all the pattern elements found in wild-type seedlings, cell shape does not seem to be relevant for the formation of the basic body pattern.

Mutations in two other genes, *knopf* and *mickey*, also change the shape of the seedling (Table 1). In contrast to *fass*, these mutations cause recognizable cellular abnormalities in certain parts of the seedling only. Mutant *knopf* seedlings look small, round and pale (Fig. 3i). Both the epidermis and the vascular strands appear affected (Fig. 4i). The epidermal cells of the hypocotyl are columnar and densely stacked, in contrast to wild-type seedlings where the epidermal layer consists of flat cells. In addition, there is no morphological indication of vascular strands in the centre of *knopf* seedlings, which instead appear to be filled with ground tissue (Fig. 4). The vascular defect, however, is not recognizable in the heart-stage embryo since the elongated cells of the vascular primordium are present in the centre (Fig. 5g), suggesting that these cells become abnormal later. The feature by which *knopf* embryos can be reliably identified at the heart stage is their broader than normal shape.

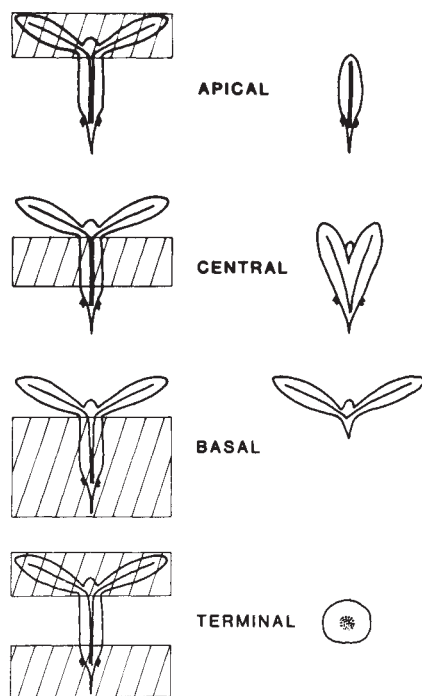


FIG. 2 Apical-basal pattern deletions. The pattern deletions caused by mutations in individual genes are schematically represented: apical (*gurke*), central (*fackel*), basal (*monopteros*), and terminal (*gnom*). The wild-type seedling patterns with the deleted parts shaded are shown on the left and the resultant mutant patterns on the right.

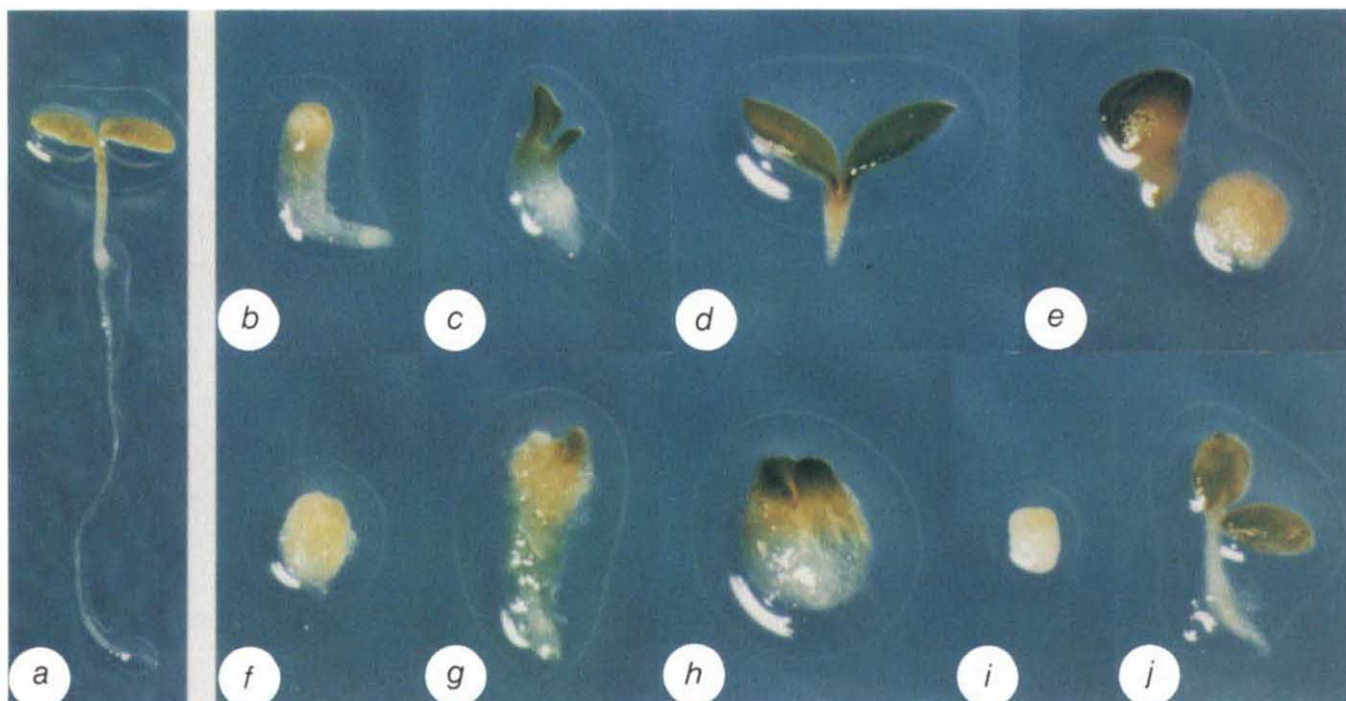


FIG. 3 Mutant seedling phenotypes. Wild type (a) on the left; top row: apical-basal pattern deletions of *gurke* (b), *fackel* (c), *monopteros* (d) and *gnom* (e); bottom row: radial pattern defects of *knolle* (f) and *keule* (g),

and shape changes of *fass* (h), *knopf* (i) and *mickey* (j). The mutant seedlings are shown at twice the magnification of the wild-type seedling.

Mutations in the *mickey* gene cause thickened disc-shaped cotyledons, which appear disproportionately large in relation to the short hypocotyl and root of the seedling (Fig. 3j). Internally, *mickey* seedlings look indistinct: not only is the texture of the cotyledons highly abnormal, but also the hypocotyl contains bloated epidermis cells and fuzzy vascular strands (Fig. 4j). This phenotype may originate in the later embryo as the heart-stage looks fairly normal (data not shown).

Discussion

The basic body pattern of higher plants is established in the embryo but is fully expressed in the stereotyped organization of the seedling. In a systematic search for pattern mutants in *Arabidopsis*, we have isolated mutant alleles of nine genes that affect three different aspects of the seedling organization: apical-basal pattern, radial pattern, and shape. Although the shape mutants do not affect pattern formation, their phenotypes can

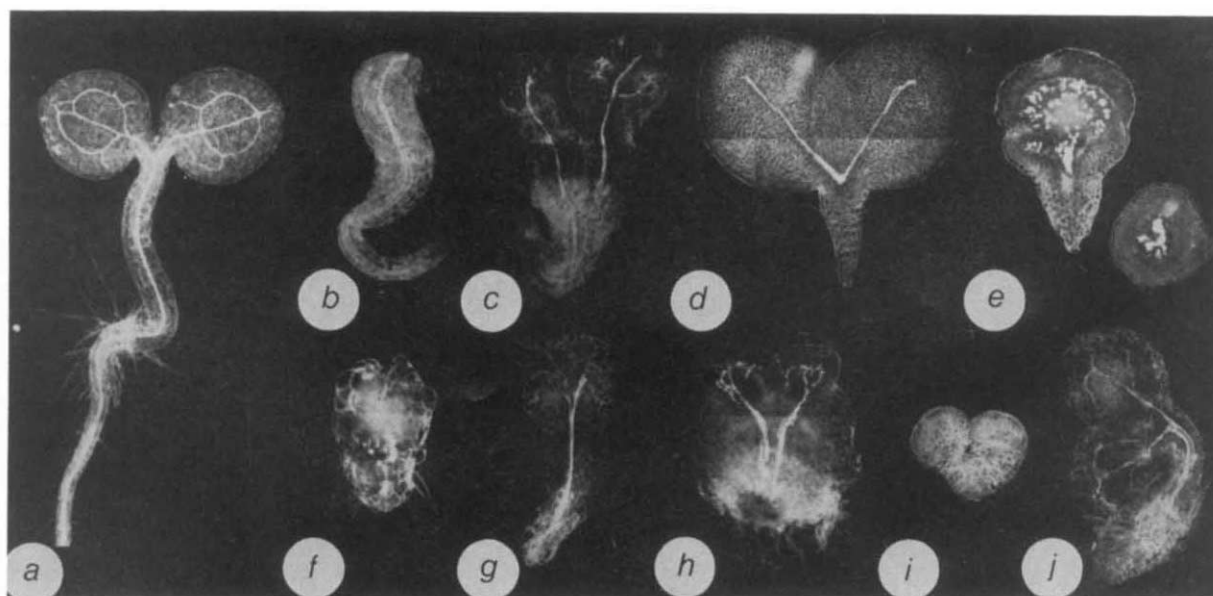


FIG. 4 Internal structures of mutant seedlings. Wild type (a) on the left; top row: apical-basal pattern deletions of *gurke* (b), *fackel* (c), *monopteros* (d) and *gnom* (e); bottom row: radial pattern defects of *knolle* (f) and *keule* (g), and shape changes of *fass* (h), *knopf* (i) and *mickey* (j).

METHODS. Seedlings were fixed in glycerol-acetic acid and mounted in Hoyer's medium (ref. 22). Whole-mount preparations were photographed with a Zeiss Axiophot $\times 10$ objective using dark-field illumination.

be used to define more precisely the conditions under which patterning genes act in the plant embryo.

Our analysis indicates that the body organization of the seedling results from two different processes in the embryo. Pattern formation generates the basic features of the organization whereas morphogenetic activities, such as localized cell divisions or cell shape changes, bring about the structure of the seedling. This distinction is evident from the study of shape mutants that display the primordia of the body pattern at the heart stage but are apparently unable to implement specific developmental programmes in response to pattern formation. Another relevant finding concerns cell shape as the morphological criterion by which to recognize, in the wild-type embryo, the primordia of pattern elements (see Fig. 1). There seem to be no primordia-specific cell shapes in *fass* mutant embryos and yet the pattern elements are present at the seedling stage. This observation strongly suggests that pattern formation is independent of cell shape and that the relative positions of cells in the early embryo determine their developmental fates. This raises the possibility that pattern formation might involve positional information¹⁰, as it does in animal embryos¹.

The seedling organization can be viewed as the superimposition of two patterns, one along the apical-basal axis and one in a radial dimension involving the primary body tissues (Fig. 1). That these two patterns are established by separate processes in the embryo is suggested by the apical-basal pattern mutants: although different parts of the axis are deleted, the remaining apical-basal pattern elements do contain all three tissues.

Apical-basal pattern formation seems to involve partitioning of the axis into three major regions: apical, central and basal. Each region is deleted by mutations in two of the four genes that specifically affect different parts of the axis (Fig. 2). Owing to the paucity of morphological features of the seedling and the variability of the mutant phenotypes, the precise boundaries of the regions cannot be determined at present. Roughly, the apical region gives rise to the shoot meristem and the cotyledons, the central region roughly corresponds to the hypocotyl, and the basal region includes the root. According to the traditional view of descriptive embryology, the primordia of the apical-basal regions as defined here are derived from the different tiers of the octant-stage embryo and the uppermost cell of its suspensor (refs 7, 11, 12; see Fig. 1). Our observations of mutant embryos are compatible with the assumption that these regions are established in the octant-stage embryo. If partitioning of the axis is

to occur at this early stage the genes involved might respond to positional information originating from the apical-basal polarity of the unfertilized egg cell¹³.

Partitioning of the apical-basal axis may be effected by the four patterning genes in either of two ways. The pairs of complementary mutant phenotypes suggest a combinatorial mode of gene action, as has been postulated for flower development^{14,15}: *gurke* and *gnom* would specify apical, *fackel* and *monopteros* central, and *gnom* and *monopteros* basal. Alternatively, these four genes may act in some hierarchical fashion with *gnom* playing a special part. The latter view rests on two observations: the *gnom* phenotype can be recognized before the octant stage (U.M. & G.J., unpublished observation), and it is highly variable, with some mutant seedlings showing no indication of apical-basal polarity. Phenotypic analysis of double mutants should enable us to distinguish between the two alternative modes of gene action.

Radial pattern formation is not well defined by the mutant phenotypes analysed. The only tissue specifically affected is the epidermis, which either does not form or develops abnormally. Our failure to isolate mutations affecting the other two tissues may be explained in two ways. One is replacement of a tissue by respecification of adjacent tissue, for example formation of new vascular strands from surrounding ground tissue¹⁶. Although we do not know whether this mechanism operates in plant embryogenesis, it may be significant that the epidermis, when experimentally removed, cannot be replaced by respecification of underlying ground tissue¹⁷. Another possibility is that drastic alterations of the radial pattern interfere with seed germination, and such mutants would have been missed in our screen (for details, see legend to Table 1). Nonetheless, the phenotypes of radial pattern mutants reveal one specific aspect: whereas the apical-basal regions by and large develop independently, the tissue layers seem to interact, and this interaction is apparently required to maintain the regular shape of the developing embryo.

On a formal level, there are interesting parallels in pattern formation between plant and animal embryos. The longitudinal body axes are partitioned into largely self-contained regions whereas the tissue layers, or germ layers of animal embryos, show interactions that influence subsequent development. Another aspect of pattern formation to be compared is genetic complexity. Our preliminary data suggest that a total of about 40 zygotically active genes may be involved in establishing the

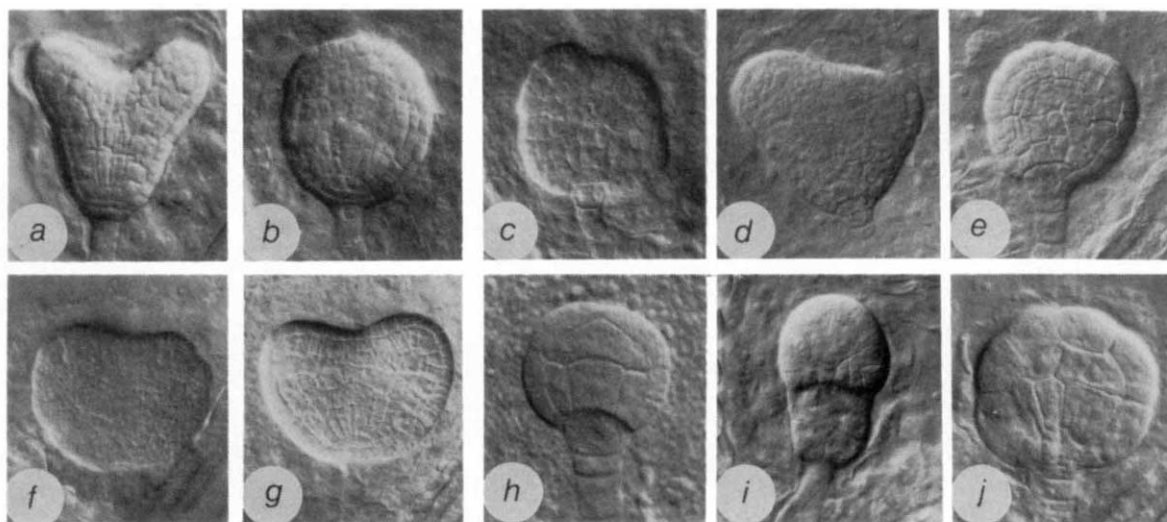


FIG. 5 Early embryonic phenotypes. Heart-stage embryos (a–g): wild type (a); apical-basal pattern deletions of *gurke* (b), *fackel* (c), *monopteros* (d) and *gnom* (e); shape changes of *fass* (f) and *knopf* (g). Globular-stage embryos (h–j): wild type (h); radial pattern defects of *knolle* (i) and *keule* (j).

METHODS. Embryos were mounted in Hoyer's medium (ref. 22). Whole-mount preparations were photographed with a Zeiss Axiophot $\times 20$ objective using Nomarski optics.

body organization in the *Arabidopsis* embryo. The only other organism in which embryonic pattern mutants have been isolated on a large scale is *Drosophila melanogaster*¹⁸⁻²¹. In both organisms, a very small proportion of the genome seems to contribute specifically to pattern formation in the embryo.

Our long-term interest lies in the analysis of how gene activities direct pattern formation in the embryo. As a first step, we have identified a number of genes whose mutant phenotypes suggest that they may play important roles in this process. There are, in our collection, other mutants with similar phenotypes to

those described here as well as mutants that have not been characterized in detail⁹. Nonetheless, the present analysis has provided a basic outline of pattern formation in the plant embryo. The apical-basal axis of polarity is partitioned into three major regions at a very early stage, probably in response to positional information derived from the polarized egg cell. The primary tissues, which appear at about the same time, originate independently of the apical-basal regions. Once the body pattern is established, differential cell proliferation and cell shape changes bring about the stereotyped organization of the seedling. □

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LETTERS TO NATURE

A kick from the solar wind as the cause of comet Halley's February 1991 flare

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KNOWLEDGE of the interaction of comets with the solar wind^{1,2} was greatly advanced during the 1986 passage of comet Halley, which was studied *in situ* and by remote sensing more thoroughly than any previous comet flyby^{3,4}. Of particular interest and novelty was the large flare⁵ that Halley was seen to produce on 12 February 1991, when it was 14.3 AU from the Sun and 18° below the ecliptic plane. There have been further outbursts, most recently on 17 March⁶. We note that the Sun has been unusually active in recent months, and suggest that a shock wave, generated by the solar flare and propagating through the interplanetary medium, could have caused the comet to flare. For example, we show that a solar flare on 31 January could plausibly have produced a shock wave that would have reached Halley on 12 February, and would have been sufficiently strong to crack the comet's crust of fluffy ice.

The propagation of solar-generated shock waves in the interplanetary medium⁷, and their interaction with planetary magnetospheres⁸ and comets^{9,10}, has been intensively investigated. Since the launches of Pioneers 10 and 11 in 1972 and 1973 and Voyagers 1 and 2 in the late 1970s, the propagation beyond several astronomical units of interplanetary shocks associated with solar flares⁷ and corotating interaction regions (CIRs)¹¹ has also been studied. Interplanetary shocks have been observed *in situ* as far in our Solar System as we have yet explored; that is, at distances greater than 40 AU as measured on Pioneer 10. Here we present preliminary analyses indicating that solar-generated shock waves (for example, from observed solar flares), and the subsequent CIR or outer heliospheric shocks that they produced may have been responsible for the recent unexpected flaring of comet Halley.

The perihelion of comet Halley occurred in March 1986, and since then the comet has been travelling towards the outer Solar System. We suggest that flaring of comet Halley in February-March 1991 was caused by a combination of circumstances. Immediately after its perihelion transit the comet still had its coma. Therefore, variations in solar activity would not have produced particularly noticeable changes. Weissman¹² attributed the post-perihelion brightening within 5 AU to seasonal effects on the obliquely rotating nucleus, whereby the sun rapidly heats the comet's northern hemisphere resulting in substantial cracking of the nonvolatile surface crust. After the comet had shed its coma to expose the fluffy amorphous crust, and the comet was dark and cold, then one of several momentum kicks from the recent shock waves generated by solar flares could have initially cracked the crust.

Figure 1 shows the February 1991 location of comet Halley in a system in which the axis from Sun to Earth is fixed, projected onto the ecliptic plane. At this time comet Halley was at a radial distance of 13.8 AU (projected onto the ecliptic) and 18° below the ecliptic plane. The general locations of Pioneers 10 and 11, Voyagers 1 and 2 and Venus (Pioneer Venus Orbiter) and ICE are also presented. There were no spacecraft located in the same quadrant of the Solar System as the comet. The angle at the Sun between Earth, Sun and comet was 143°. Nevertheless, it is useful to examine the indices for solar activity, as active regions and coronal holes on the sun can persist for significantly longer than half a solar rotation. Their effects, therefore, could be observed at Earth and then, as the Sun rotates, about 10 days later at the longitude of the comet. Also, the longitudinal extent of solar-generated shock waves increases as they propagate away from the Sun.

The H α synoptic charts of the sun (World Data Center A for Solar Terrestrial Physics, personal communication) indicate that in January-February 1991 there was a large northern polar coronal hole extending toward the heliographic equator, but there were no significant southern coronal holes. Thus, no coronal hole is a candidate source for a solar generated shock and a high speed stream that ultimately could have given rise to the flaring of comet Halley on February 12. Other candidate sources are associated with active regions and flares on the sun. For example, flares, characterized by radio Type II onsets, occurred on January 25, 27, 30 and 31 (World Data Center A

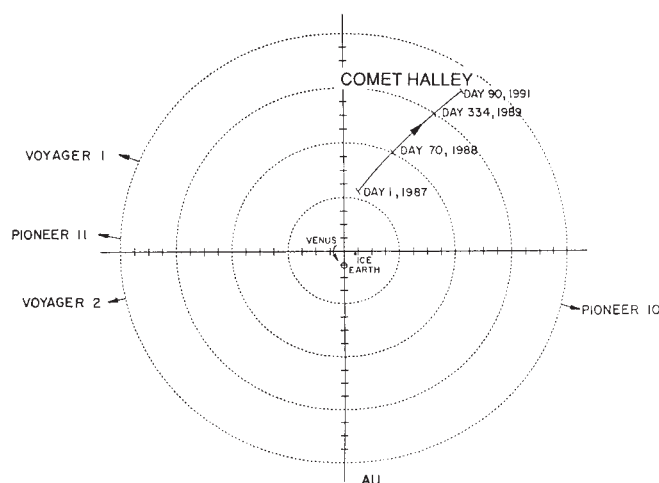


FIG. 1 Location of Halley's comet, projected into the ecliptic plane, in a system with fixed Sun-Earth axis. On 12 February 1991 (day 43) the comet was at a heliocentric radial distance of 14.3 AU and 18° below the ecliptic plane.

for Solar Terrestrial Physics, personal communication). These major flares, classified by their soft X-ray and optical characteristics given in Table 1, were located in the southern solar hemisphere. The locations and classifications of other representative large flares are also given.

The current maximum of solar cycle 22 (late 1990 into 1991) has been characterized by a number of large solar flares. Both the size (as observed in $H\alpha$) in the 3B category (where B stands for bright), for example, and the X-ray emission (greater than 10^{-4} W m^{-2}) of these flares indicate that they merit the term 'large'. Flares of these magnitudes release more than 10^{32} erg. By comparison, a 1-MT nuclear bomb is equivalent to 10^{23} erg. Table 1 gives a representative list of large flares during the potential period of interest. Their locations on the visible solar disk are listed relative to a fixed Sun-Earth axis. Initial shock velocity may be estimated from the radioastronomical drift (frequency against time, coupled with a coronal density model). The time duration of the X-ray emission is taken to indicate the duration of constant-velocity, piston-driven shock motion¹³, after which the shock velocity is taken to decay as $R^{-1/2}$, where R is the heliocentric radius (as does the shock in a nuclear bomb blast). This information is important if we are to estimate the time and geometry of shock arrival from any of these flares (especially those 'favourably' oriented toward P/Halley). We will suggest that blast loadings on the icy crust ($\sim -200^\circ\text{C}$) of the nucleus of the comet caused it to crack, allowing dust to be driven away from the surface and, thereby, temporarily replenishing the gas and dust that earlier had dispersed into space before, say, December 1990–March 1991.

The kinematics of the shock from the flare on 31 January 1991 are taken as a typical example of a large flare during this maximum of solar cycle 22. The discovery of the cometary outburst on 12 February 1991 may have been a coincidence, as a new observing run was begun during the new moon when Halley was most favourably displaced for observation. In principle, the outburst may have started 1–2 months earlier, but we are not aware of any published data to this effect. A single press report (*Science News*, 15 June 1991) noted a speculation that the outburst occurred during the third week of December 1990. If this is proved to be valid, then the physical explanation summarized below would have to be ascribed to a flare earlier than our example. Thus, there are still many sources of uncertainty in our model, such as the actual time of outburst, conditions of the amorphous icy crust (thickness, modulus of elasticity, failure point due to transient pressure loading and increased drag effects), trajectory of the flare shock and strength

TABLE 1 Representative major solar flares (at/near maximum of cycle 22)

Date	Location on solar disk	X-ray/optical classification
24 November 1990	$04^\circ \text{S } 04^\circ \text{E}$	M3/1B
10 December	$15^\circ \text{N } 47^\circ \text{W}$	M6/2B
12 December	$19^\circ \text{N } 74^\circ \text{W}$	M2/SF
15 December	$12^\circ \text{N } 35^\circ \text{E}$	M2/1N
23 December	$11^\circ \text{N } 68^\circ \text{W}$	X1/2B
25 January 1991	$16^\circ \text{S } 78^\circ \text{E}$	X10/SF
27 January	$4^\circ \text{S } 59^\circ \text{E}$	X2/1B
30 January	$08^\circ \text{S } 34^\circ \text{W}$	X1/2B
31 January	$17^\circ \text{S } 35^\circ \text{W}$	X13/2B

at impact, and identification of the likeliest strong flare and its shock. Figure 1, for example, shows that P/Halley (at 14.3 AU heliocentric distance; 13.8 AU, when projected, as shown, on the ecliptic plane) was $\sim 143^\circ$ to the west of the Sun–Earth axis.

Any flares that were in the best geometrical position (with respect to the strongest portion of their expanding shock fronts) could not be directly observed from Earth. The flares listed in Table 1 were, of course, duly recorded at Earth. One of these candidates is the flare of 31 January 1991, assuming that the cometary outburst on 12 February 1991 was actually the first outburst since the demise, several years earlier, of its dusty, gaseous coma.

With these caveats in mind, it is instructive to consider the radio Type II onset at 0230 UT on 31 January 1991. From the above procedure, we can estimate an initial shock speed of $3,000 \text{ km s}^{-1}$ which lasted for 6 h (this is the duration of the proxy X-ray emission). The location of the solar flare at $17^\circ \text{S } 35^\circ \text{W}$ (projected into the ecliptic plane), relative to the axis connecting comet Halley to the Sun, is shown by a small, downward-pointing tick mark in Fig. 2. Units of 1 AU on the horizontal axis ending at 13.8 AU at P/Halley (labelled 'comet') are indicated by the larger vertical tick marks.

The model of shock propagation for this case indicates that the Mach number was 6.1 when it reached P/Halley on 21 February 1991, nine days later than the first observation of its outburst. We do not consider the 'judicious' adjustment of the

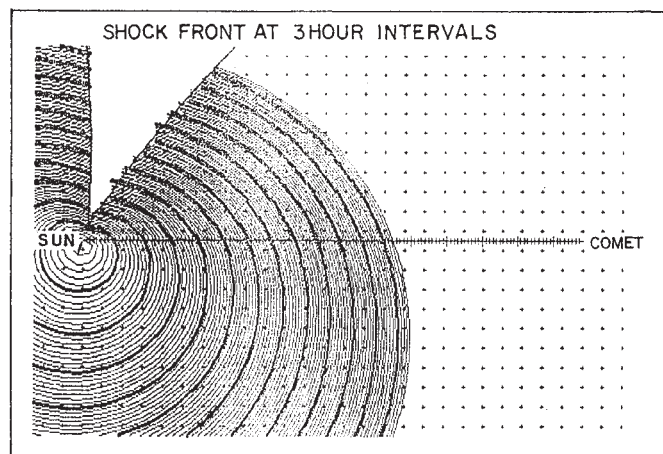


FIG. 2 Example of strong interplanetary shock generated by a solar flare propagating toward P/Halley (labelled 'comet' at the end of the horizontal axis in the ecliptic plane). In this example, the responsible flare generated a shock on 31 January 1991 with a shock speed (inferred from radio Type II emission) of $3,000 \text{ km s}^{-1}$ at 0230 UT. The flare location, relative to the axis connecting Halley to the Sun, is shown by a small, downward-pointing tick mark (just to the right of the word 'SUN'). The shock's location is shown every 3 h for a representative 12-day duration (dark lines). The larger vertical tick marks on the horizontal axis indicate units of 1 AU. The V-shaped open zone at the top of the figure indicates the region where the shock decayed into a weak (hence ineffective) magnetohydrodynamic wave.

variables involved (such as uncertainties of constant velocity duration near the sun, a higher initial velocity, background solar wind velocity) to be justified for the purpose of this estimate.

The blast loading on an object is a function of both the incident blast wave characteristics (the peak overpressure, dynamic pressure and decay of pressure (drag) loading behind the shock) and of its duration. The overpressure on the front face can be found from dynamic shock-on-shock calculations (Fig. 6 of ref. 8) to be $P/P_T = 28$ for Mach 6 and a specific heat ratio of 5/3. Here, P_T is the dynamic pressure at the stagnation point of the cometary object before impact. At 14.3 AU, this pressure is $\sim 10^{-10}$ dyn cm $^{-2}$. Hence the peak overpressure, which lasts for ~ 2 ms (see Fig. 5 of ref. 8 for a comparison of laboratory and numerical simulation results), is $\sim 3 \times 10^{-9}$ dyn cm $^{-2}$. The drag loading, after the peak pressure duration, is about half this value (again, see Fig. 6 of ref. 8) but lasts for many hours after shock propagation into the anti-solar direction. The initial impact loading [3×10^{-9} dyn cm $^{-2} \times \pi(6 \text{ km})^2$] is $\sim 3,400$ dyn on the roughly hemispherical face of P/Halley. Experience with nuclear explosions shows that a visible (that is, apparent) crater is accompanied by a rupture zone and a plastic zone (Gladstone¹⁴, Ch. VI). In the case of a fluffy amorphous icy crust on Halley (depth unknown), fissures, if not outright cracking, are possible. We are not aware of any published data on the compressive failure of fluffy amorphous ice at this low temperature (-200°C). Associated with this scenario may be the heating suggested by Smoluchowski¹⁵. The temperature rise associated with the shock-on-shock impact would possibly cause some transformation from the amorphous to the crystalline state. Part of the impulsive pressure increase referred to above (a factor of 28) is due to the increase in density of the solar wind (a factor of 4). Thus, the combination of the temperature increase (a factor of 7) with the mechanical impact is likely to produce crystallization. The pressure of outflowing gases ($\sim 10^3$ dyn cm $^{-2}$) then dominates after the fissures occur¹⁶.

Another issue to be addressed is whether there were comparable or greater increases in the solar wind pressure at the comet before December 1990–March 1991. These increases could have weakened the crust. We examined the available data on the solar wind from the Pioneer Venus Orbiter (PVO) from September 1990 to March 1991. In November 1990, PVO was at superior conjunction, and tracking of the spacecraft was therefore sparse. In addition, the competition for tracking was high from September to January, so that the tracking coverage of the spacecraft was low. Nevertheless, solar wind speeds are available on most days. From September to December 1990, the solar wind speed, as measured by the plasma analyser on PVO, was ≥ 600 km s $^{-1}$ on only five occasions. From January to 10 April 1991, the speed was ≥ 600 km s $^{-1}$ on nine occasions. During the latter period, PVO was located $\sim 180^\circ$ away from comet Halley (see Fig. 1); nevertheless, these observations indicate that one or more strong flare-generated shock waves probably propagated outward through a quiescent solar wind and provided a strong enough impulse to cause the flaring of comet Halley. $\square$

Structure and growth mechanism of mineral dendrites

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THE surfaces of limestones are often marked by black or red-brown deposits known as mineral dendrites^{1,2}. These are deposits of hydrous iron or manganese oxides formed when supersaturated solutions of iron or manganese penetrate the limestone and are precipitated on exposure to air at the surface. Mineral dendrites have a fractal³ appearance, but the origin and characteristics of this morphology, and its dependence on concentration gradients or reaction rates have been little studied. Here we analyse the shapes and fractal properties of mineral dendrites from several different origins, and propose a lattice reaction–diffusion model for their formation. The model shows how different reaction conditions can account for the variation in fractal dimension observed in real dendrites.

Mineral dendrites are distinct from the ‘dendritic’ crystal morphologies obtained by solidification from an undercooled melt, the understanding of which is fairly well developed^{4,5}. Mineral dendrites are formed by an irreversible chemical reaction and have an appearance similar to the clusters of diffusion-limited aggregation (Fig. 1) rather than the branched form of ‘dendritic growth’^{4,5}.

We collected samples of mineral dendrites from various parts of the world and photographed them. For those samples with good contrast, we digitized the pictures with a resolution 720×600 and analysed them using the sand-box technique⁶ to calculate the fractal dimension. This method is based on determining the mass M (the number of occupied pixels of the digitized image) within square boxes of varying size centred at points randomly selected on the fractal pattern. The fractal dimension d_f is obtained from the expression $M(r) \propto r^{d_f}$, where $M(r)$ is the average mass in boxes of linear size r . We selected 2,000 centres for each picture.

The results for three different samples are shown in Fig. 1. The first sample, from Bavaria (Fig. 1a), consists of particularly well-developed, densely packed long branches which can be distinguished with a resolution of <0.1 mm. The second sample, from Greece (Fig. 1b), shows more common, shorter, but also fairly densely packed dendrites emerging from cracks in limestone. For both Fig. 1a and 1b, results are shown for two different samples from the same location. If the mass within a box is plotted logarithmically against the size of the box (left-hand side of Fig. 1), we find straight lines of non-integer slope over about two orders of magnitude, showing that the patterns are fractal. The fractal dimension d_f can be read off and we find for the first two samples $d_f = 1.78 \pm 0.04$. In the third sample, from Brazil (Fig. 1c) the dendrite is an inclusion within the plane of a crack inside a quartz crystal. There is only one dendrite, probably because there was a single injection hole. It is less dense, but is fractal, and the fractal dimension obtained from the figure is $d_f = 1.51 \pm 0.05$. We have analysed in this way other samples with finger widths (length scale) ranging from one-tenth to several millimetres. All are fractal. Those on limestone are dense, with fractal dimensions of ~ 1.78 ; in quartz, their fractal dimension is lower.

To explain the dendritic structures, we will mimic the chemical process of manganese ions diffusing out of cracks and oxidizing in the reaction: $\text{Mn}^{4+} + 2\text{O}^{2-} \rightarrow \text{MnO}_2$. For this we devised the following reaction–diffusion model. We consider two species of particles, A and B, which diffuse on a square lattice. When an A and a B particle are simultaneously on the same site, they react forming C ($A + B \rightarrow C$). The C particles also diffuse until they precipitate into D ($kC \rightarrow D$). The D particles are stationary, and the sites where they are created will be marked black for

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all successive time-steps (see Fig. 2a-c).

Two conditions will lead C to transform into D: first, when at least k particles of type C simultaneously meet on the same site, saturation occurs, and the C particles precipitate to form D; second, when a C particle becomes nearest neighbour to a black site D, it aggregates to the cluster.

Such a reaction-diffusion process will cause a reaction front to build up if the A and B species are initially separated in space. If the concentrations of A and B (ρ_A and ρ_B) are different, this reaction front moves across the system, leaving clusters of D particles in its wake. The values of ρ_A and ρ_B play a crucial part in the dynamics, as they determine the concentration of C particles produced in the reaction zone and the speed of the

front (which moves faster as the concentration difference increases).

Because of the motion of the front, the clusters grow asymmetrically. Their density depends on the amount of C released by the A-B reaction. If the rate at which C particles aggregate is not too large compared with the rate at which they are produced, long and continuous clusters are formed along the direction of motion of the front. On the other hand, if the C particles are produced too slowly and the front moves fast enough, the cluster growth will die out rapidly because all the C available at a given time and position will be exhausted by aggregation. To create a new cluster, one has to wait until spontaneous precipitation occurs ($kC \searrow D$), which requires a

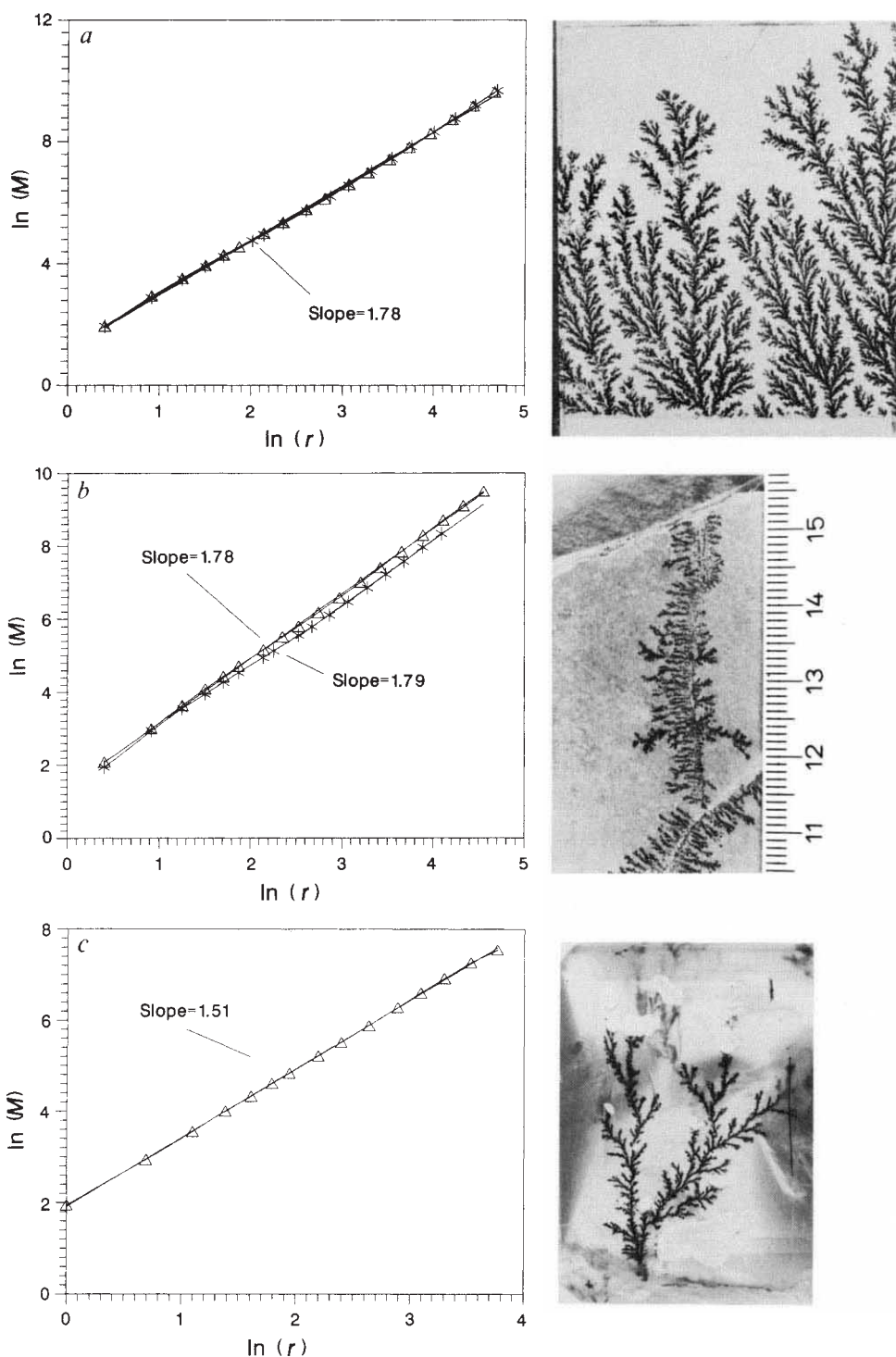


FIG. 1 Photographs of various manganese oxide mineral dendrites (left) and the log-log plots (natural logarithm) of their mass as function of the length of the box (right) for *a*, Solnhofener Kalkstein, Bavaria; *b*, limestone from Corfu, Greece and *c*, inclusions in quartz from Ouro Preto, Brazil.

large enough concentration of C. This is likely to happen after the reaction $A + B \rightarrow C$ has run for a while, that is at some position further away. This gives rise to small and isolated clusters.

The numerical simulation is set up on a two-dimensional square lattice, with periodic boundary conditions in the horizontal direction. The system is initially filled with a concentration $\rho_A = 1$ of A particles on the lower part and a concentration $\rho_B \ll 1$ of B particles on the upper part. On the bottom line, there is a source of A particles with a concentration of 1: on each site, any particle that leaves is immediately replaced by a new one. On the top line, a source of B with concentration ρ_B is similarly imposed. We chose $k = 4$ as the number of C particles needed to form a precipitate.

Because of the externally imposed concentration gradient $\rho_A \gg \rho_B$, the reaction front moves upward leaving behind it a tree-like pattern. The smaller the concentration ρ_B , the faster the reaction front moves and the less dense are the precipitate clusters left behind, as seen by comparing Fig. 2a ($\rho_B = 1/20$) with Fig. 2c ($\rho_B = 1/50$). In Fig. 2b, we put straight lines of precipitate into the lattice by hand before starting the diffusion process, to mimic the fracture lines seen in Fig. 1b. The structures of Fig. 2a–c strongly resemble the pictures in the corresponding Fig. 1a–c, indicating that our reaction–diffusion model describes the formation of mineral dendrites fairly well. A similar reaction–diffusion scheme is also believed to be responsible for the formation of the ‘Liesegang bands’^{7,8}. Although some features of these bands are captured by our model, the time and length scale are not appropriate.

Our numerical algorithm is of cellular automaton type. It is well suited for a vector computer or a massively parallel architecture. Our simulations were conducted both on a CRAY-YMP and a 16-K Connection Machine. The simulations were run for $\sim 10^5$ time steps, over a lattice of 512×512 sites. Only a fraction of the total size ($\sim 1/4$ and $1/8$) is shown in Fig. 2. The speed of the algorithm is $\sim 2 \times 10^7$ updates per second, on both machines.

We next analyse the fractal dimension of our cluster. We use the sand-box method by placing concentric boxes, of linear size 2–20 lattice sites, so that they are centred around sites of the precipitate (see Fig. 2a–c). A log-log plot of the mass inside a box (number of black sites) as a function of its size produces the straight lines shown in Fig. 2d, f and g, corresponding to the clusters of Fig. 2a, b and c, respectively. In the high-density cases, where $\rho_B = 1/20$ (Fig. 2d and f), we find $d_f = 1.76$, whereas $\rho = 1/50$ gives the lower value of 1.58 (Fig. 2g). To check the reliability of the method, we also analysed the pattern of Fig. 2a using box-counting⁶: we placed grids of different lattice sizes over the system and counted for each size the number of boxes that are not empty. A log-log plot of these data should give a straight line with slope $-d_f$. The results are shown in Fig. 2e; when the smallest boxes are excluded, the value of d_f obtained in this way is consistent with that obtained from the sand-box method.

The fractal dimensions calculated from our numerical model agree well with those obtained from the analysis of real mineral dendrites. We conclude from this that the model describes the essential features of the process underlying the formation of dendrites in nature: diffusion, with a reaction front and irreversible aggregation. These elements are also responsible for the formation of fractals in electrodeposition of zinc leaves⁹ and other experimental applications of diffusion-limited aggregation (DLA)¹⁰. But DLA in its original form cannot explain all features of mineral dendrites. First, we know that in our case the chemistry is more complex, and the existence of at least three species has to be considered. Second, the observed dendrites have varying fractal dimensions, whereas DLA results in a unique value $D \approx 1.70$. Our process is similar to reaction-limited aggregation with the difference that in our case the reaction takes place in a moving front.

We conclude that on a certain length scale, mineral dendrites

are fractal, and that this effect can be explained by a reaction–diffusion–aggregation model which has similarities to other laplacian growth models that produce fractals⁶. The range over which the dendrites are fractal is limited from below by the finger width and from above by the size of a tree and covers easily two orders of magnitude. In this sense our fractal dimensions are only effective exponents, and their value depends on the density of the precipitate. For the more typical case of manganese on limestone, a dimension of ~ 1.75 is found, whereas inclusions in crystals have lower values. Probably these exponents can be explained as a crossover behaviour towards the critical case of infinitely large clusters. Although the numerically obtained pictures are very similar to the photographs of

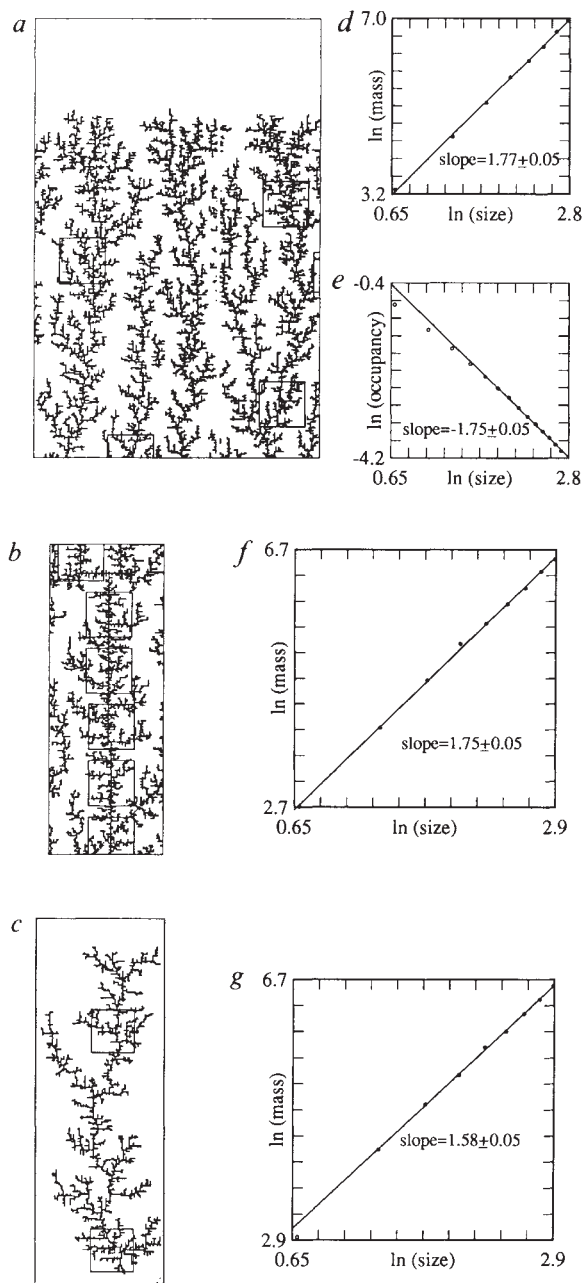


FIG. 2 Numerical results from the reaction–diffusion model. The initial concentration of B is $\rho_B = 1/20$ in a and b, and $1/50$ in c. The measurement of the fractal dimension with the sand-box method is shown in d, f and g for the clusters obtained in a, b and c, respectively. The square boxes in a, b and c show the maximum box size used for this measurement. The results of a box-counting method for a are given in e. Only the data shown as black dots have been taken into account in the least-squares fit.

mineral dendrites, the underlying square lattice is still recognizable in the simulated precipitates. Therefore, off-lattice simulations would be interesting. □

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Spatial orientation of molecules in strong electric fields and evidence for pendular states

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IN typical collisional or spectroscopic experiments, molecules rotate freely with random spatial orientations. The resulting isotropic averaging obscures or suppresses much stereodynamical information and has remained a recalcitrant problem. The only practical means for orienting a molecule itself, rather than just its axis of rotation, has been electric field focusing^{1,2}. But this is applicable only to certain rotational states of symmetric top molecules (or equivalent) that exhibit a first-order Stark effect. Orientation of molecules other than symmetric tops has long been considered to be quite unfeasible³. Recently, however, it has been pointed out^{4,5} that by exploiting the extreme rotational cooling that can occur in supersonic molecular beams, substantial orientation of diatomic, linear or asymmetric top molecules should become possible at accessible field strengths. The anisotropy of the Stark effect allows molecules in the lowest few rotational states to be trapped in 'pendular states' and thereby confined to librate (oscillate about the field axis) over a limited angular range. Here we describe an experiment which demonstrates that oriented pendular states can be obtained for a diatomic molecule with modest field strengths. With anticipated improvements, this technique should become widely applicable.

The ability to orient symmetric top molecules has enabled studies to be carried out to probe the anisotropic forces governing collisions with reactive atoms^{6–8}, photons⁹ or surfaces¹⁰. In contrast to symmetric tops, however, which in the orientable states precess about the field vector, diatomic, linear or asymmetric top molecules tumble in all rotational states. This averages out the dipole moment in first order and hence greatly weakens interaction with an electric field. Typically, for a gas at room temperature, the interaction energy of the dipole moment with a feasible electric field is only ~0.1–1% of the mean rotational energy. Thus despite the presence of the field, all but a very small fraction of the molecules continue rotating like pinwheels.

A molecular beam formed by supersonic expansion offers a great advantage (see ref. 11 and refs therein) as collisional relaxation can drop the rotational temperature down to 1 K or less. Theoretical calculations^{4,5} show that for many polar molecules, such cooling should permit a large fraction of the beam molecules to be considerably oriented without need for inordinately large field strengths. The cooling condenses much of the beam population into the lowest few rotational states.

These can be converted from pinwheeling to pendular states simply by sending the beam into a region subject to a strong uniform electric field. For most prospective applications, this region can be quite small; there is no need for the long focusing fields used to select the orientable states of symmetric tops.

In our experiment a molecular beam containing ~15–20% ICl seeded in H₂ is formed by expanding the gas mixture at 110 torr and 310 K through a glass nozzle 250 μm in diameter. The beam passes between a pair of parallel plate electrodes, 2 cm long, 2 cm wide and 1.7 cm apart, fashioned either from metal grids or from conducting glass (a glass with a conducting transparent layer of fluorine-doped SnO₂ was provided by R. Gordon). In the gap between these electrodes, the molecules are subject to a static electric field which could be scanned up to 20 kV cm⁻¹. The molecular beam within the gap is illuminated with orange-red light from a pulsed dye laser (Lambda Physik, EMG 202/FL 3002E). By scanning the laser wavelength, we observe an excitation spectrum with resolved rotational structure. This is detected by a photomultiplier (RCA C31034A) which views the accompanying optical fluorescence through one of the electrodes. Active background subtraction and gating of the photon counters is used to enhance the signal-to-background ratio. The field-induced shifts in rotational line positions and intensities provide the means to characterize the extent of molecular orientation achieved.

Figure 1 shows examples of laser-induced fluorescence spectra, illustrating striking changes in the rotational structure with the field strength. The particular vibronic band of I³⁵Cl was chosen for study because of its favourable Franck-Condon factors and little overlap by lines from I₂ contamination in the beam. Accurate spectroscopic constants are well established¹² for the ground electronic state, X¹Σ⁺. Less accurate constants, including the dipole moment, are also available for the A³Π excited electronic state from the work of Cummings and Klemperer¹³ and others^{14–16}. We obtained spectral assignments and values of the dipole moment μ' and rotational constant B' consistent with their results.

Figure 2 compares the lowest few energy levels¹⁷ and transitions with (left) and without (right) the electric field. The states are labelled with quantum numbers J, M, designating the rotational angular momentum and its projection on the field direction. Transitions are labelled in the usual fashion (see Fig. 1). We also plot (left) the potential energy, given by -με cos θ, for a dipole moment μ in an electric field of strength ε, with θ the angle between the dipole axis and the field direction. The energy levels bound within the cosine potential correspond to pendular states. These states are hybrids of the field-free rotational states^{18,19}, comprising linear combinations of spherical harmonics with a range of J but the same fixed value of M. With increasing field strength, the range of J contributing to a pendular state expands as the state is drawn down lower in the cosine potential and its librational amplitude narrows.

For the ICl spectra examined here, the dipole moment of the excited electronic state A³Π is similar in magnitude but suspected¹³ to be opposite in sign to that for the ground state, X¹Σ⁺. Reversing the sign of the dipole does not change the energy levels but is equivalent to shifting the cosine potential by θ → θ + 180°. This is advantageous for our purpose. For example, if the laser-induced excitation of an ICl molecule occurs with θ ≈ 0°, this will correspond to the most attractive region of the potential well for the ground state X but to the most repulsive region for the excited A state. Accordingly, the excitation probability for a rotational transition such as R₀₀, corresponding to 0, 0(X) → 1, 0(A), will depend on the exponentially decaying tail of the 1, 0(A) wavefunction in the classically excluded region, once the field strength becomes large enough to ensure that both 0, 0(X) and 1, 0(A) are pendular states. This makes the intensity of such transitions extremely sensitive to the librational amplitude.

We find that all aspects of the field-induced spectra conform to the theoretically predicted behaviour. As seen in Fig. 2, the librational amplitude at the highest field strength used in this experiment is substantially less than $\pm 90^\circ$ only for the $0, 0(X)$ and $0, 0(A)$ states. For most other states, the classical turning points are at much wider angles. Particularly for the $J, 0$ states with $J > 0$ but $M = 0$, the dipole perversely spends most of its time opposed to the field. These properties are evident in the magnitude and direction of the Stark shifts $\Delta\nu$ relative to the

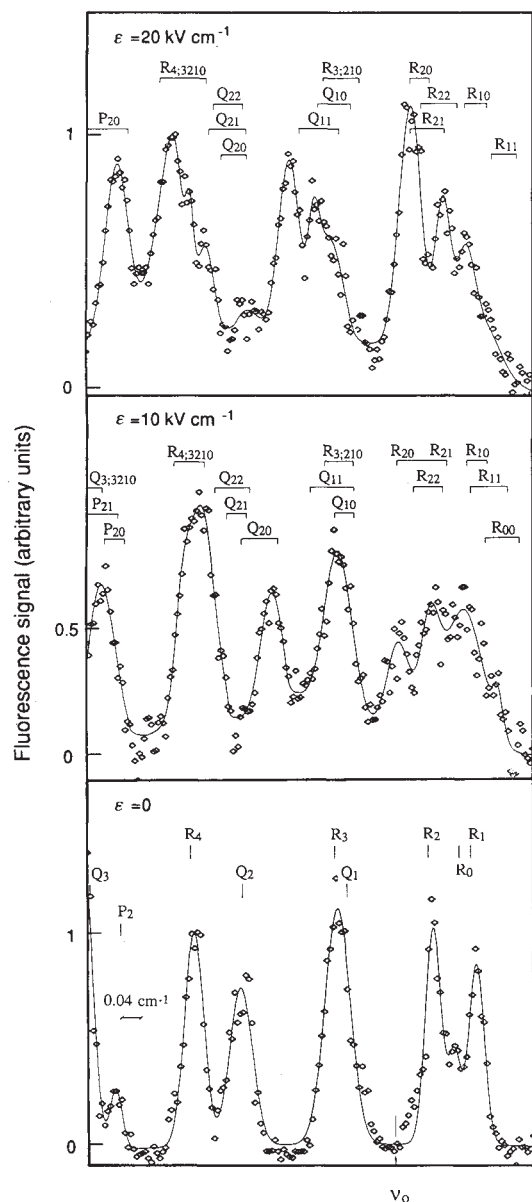


FIG. 1 Excitation spectra for ICI seeded in a supersonic beam of H_2 , from $v''=0 \rightarrow v'=19$ vibrational band of the $X^1\Sigma \rightarrow A^3\Pi$ electronic transition, at field strengths $\epsilon = 0, 10$ and 20 kV cm^{-1} obtained with laser radiation linearly polarized parallel to the electric field ϵ . Resolution $\sim 0.04 \text{ cm}^{-1}$. Band origin (for $\epsilon = 0$) at $\nu_0 = 16,652.709 \text{ cm}^{-1}$ (ref. 14). Scan from $\nu_0 - 0.623 \text{ cm}^{-1}$ (left) to $\nu_0 + 0.277 \text{ cm}^{-1}$ (right). Full curves are gaussian fits to the experimental points. Rotational transitions $J'', M'' \rightarrow J', M'$ are labelled (see Fig. 2) as usual: R, Q, P indicate $\Delta J = J' - J'' = +1, 0, -1$, respectively, all with $\Delta M = 0$; subscripts indicate the initial J'' (and also M'' when $\epsilon > 0$). Theoretically predicted line positions are indicated, as calculated from molecular parameters (electric dipole moment $\mu'' = 1.24$, $\mu' = 1.25 \pm 0.15$ debye units; $B'' = 0.114156$, $B' = 0.06206 \pm 0.0005 \text{ cm}^{-1}$); uncertainty is mostly due to that in upper-state dipole moment. Rotational temperature of ICI is $\sim 15 \text{ K}$.

field-free transition frequencies. More revealing are the expectation values of the orientation cosine, $\langle \cos \theta \rangle$, which provide a measure of the extent of orientation. By applying the Hellman-Feynman theorem⁵ to the field-dependence of the Stark shift, we can evaluate the difference $\Delta \langle \cos \theta \rangle$ for the pair of states involved in each transition from the equation $\partial \Delta\nu / \partial (\mu\epsilon) = -\Delta \langle \cos \theta \rangle = \langle \cos \theta \rangle_X - \langle \cos \theta \rangle_A$, with $\mu' \approx \mu''$ here. Figure 3 compares, for the R_{00} , R_{11} and Q_{21} transitions, the experimental values of these differences $\Delta \langle \cos \theta \rangle$ with those obtained from theoretical calculations for the individual states. As the Q_{21} transition involves states only slightly perturbed by the electric field (see Fig. 2), $\Delta \langle \cos \theta \rangle$ remains close to zero, the isotropic value. The R_{00} and R_{11} transitions, however, involve pendular states and $\Delta \langle \cos \theta \rangle$ reaches values considerably different from zero. Likewise, comparison of the observed and predicted intensity variations of the Stark shifts provides striking evidence for pendular states. For instance, the intensity of the R_{00} transition drops by $\sim 50\%$ at $\epsilon = 10 \text{ kV cm}^{-1}$ and practically vanishes by $\epsilon = 20 \text{ kV cm}^{-1}$.

Experiments using molecules in pendular states must be conducted in a strong electric field, sufficient to maintain the hybridization of rotor states that produces the angular localization. This requirement will for some purposes be awkward

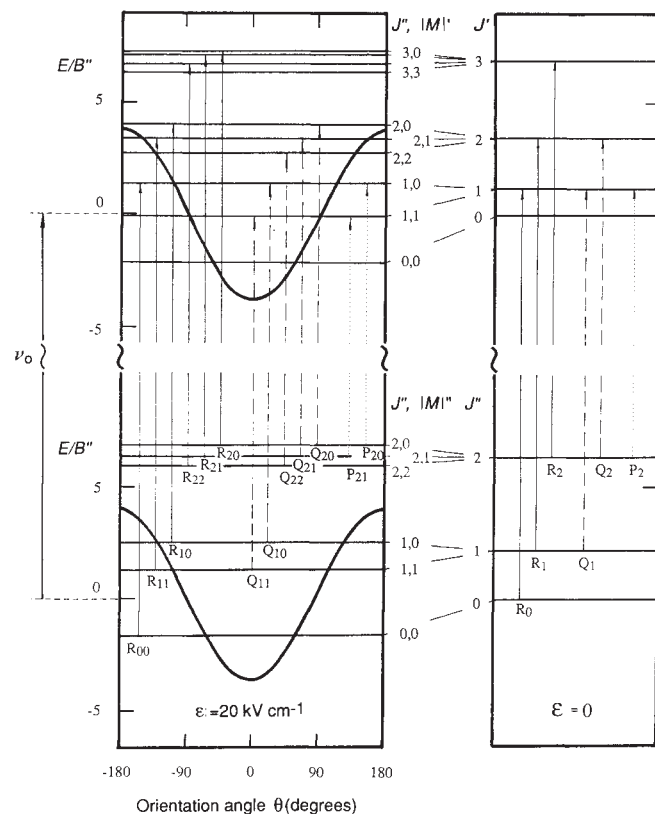


FIG. 2 Rotational energy levels for ground ($X, v''=0$) (lower half) and excited ($A, v'=19$) (upper half) vibronic states and transitions, calculated from molecular parameters (see Fig. 1 for numerical values and labelling). The excited A state has nonzero electronic angular momentum, but for simplicity this is omitted in these computations; thus the rotational states are labelled with the nominal J', M' values for a bar-bell rotor. Right-hand panel applies to field-free case, left-hand panel to highest field strength in present experiment, $\epsilon = 20 \text{ kV cm}^{-1}$. Ordinate energy scale is in units of the ground-state rotational constant, B'' . Also shown are the Stark interaction potentials for both the X and A states, proportional to the cosine of the angle θ between the dipole moment μ and the field direction. For the A state, the change in sign of the dipole moment (see text) does not alter the energy levels but displaces the interaction potential by $\theta \rightarrow \theta + 180^\circ$; for simplicity this displacement is not shown. At $\epsilon = 20 \text{ kV cm}^{-1}$, the Stark potential for X binds three pendular states and that for A binds five.

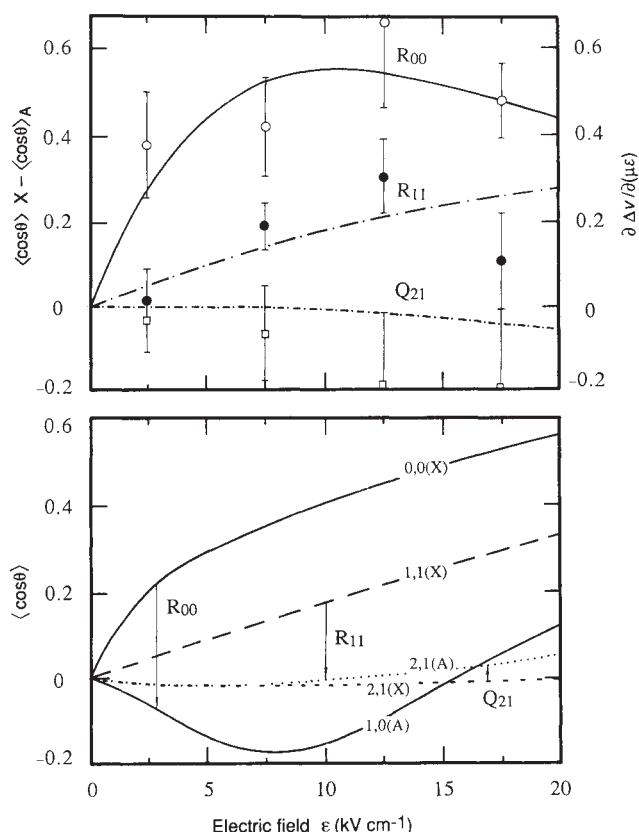


FIG. 3 Variation with the field strength ϵ of the expectation values of the orientation cosine, $\langle \cos \theta \rangle$, for individual states (lower panel) and for differences, $\langle \cos \theta \rangle_X - \langle \cos \theta \rangle_A$, pertaining to the R_{00} , R_{11} and Q_{21} transitions (upper panel). Curves are derived from Stark effect calculations, points from observed spectra. As described in text, the orientational differences, $\langle \cos \theta \rangle_X - \langle \cos \theta \rangle_A$, are evaluated from derivatives of the field-induced shifts Δv with respect to the Stark interaction energy $\mu \epsilon$.

or prohibitive, but many experiments can accommodate or even exploit the presence of a strong field. For instance, collisions of oriented molecules with atoms or molecules can be studied by sending the beam through suitable grids. Likewise, spectroscopy and even electron diffraction can be carried out with modest alterations. For molecules containing quadrupolar nuclei, a strong field suppresses unwelcome complications, by uncoupling the nuclear spin from molecular rotation. Especially attractive is the opportunity to observe spectroscopic transitions as the molecular orientation is scanned relative to the polarization of the electric vector of the radiation. As well as suppressing rotational averaging, the orienting field can be used to tune the pinwheel/pendulum levels. Strong-field Stark shifts equivalent to a fivefold or larger change in rotational constant are attainable. This could prove useful in many current experiments involving multiphoton processes. For instance, transitions requiring two resonant photons of different colour could instead be brought about with a pair of one-colour photons, by tuning the energy levels.

In this diagnostic experiment, we have aimed only to confirm the feasibility of an extremely simple means for orienting molecules. The apparatus and technique employed here are far from optimal; in particular, field strengths tenfold higher have been attained in electric deflection studies²⁰. With such fields, the hybridization of rotor states would be far more pronounced, and much better orientation could be obtained for many molecules. Because of its simplicity and compactness, the technique could be applied in a wide range of experimental situations. □

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Sonochemical synthesis of amorphous iron

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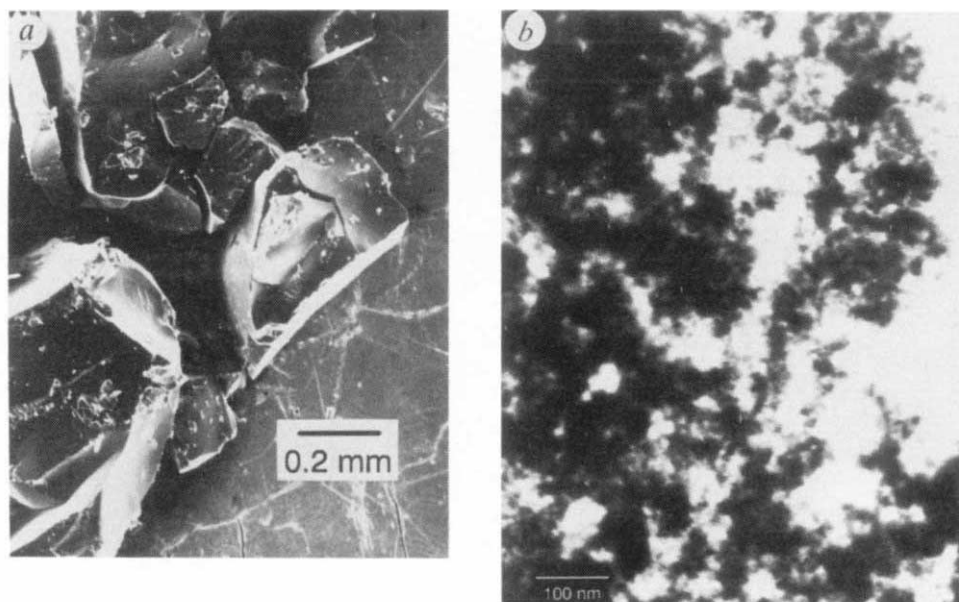
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AMORPHOUS metallic alloys ('metallic glasses') lack long-range crystalline order and have unique electronic, magnetic and corrosion-resistant properties^{1–3}. Their applications include use in power-transformer cores, magnetic storage media, cryothermometry and corrosion-resistant coatings. The production of metallic glasses is made difficult, however, by the extremely rapid cooling from the melt that is necessary to prevent crystallization. Cooling rates of about 10^5 to 10^7 K s⁻¹ are generally required; for comparison, plunging red-hot steel into water produces cooling rates of only about 2,500 K s⁻¹. Metallic glasses can be formed by splattering molten metal on a cold surface using techniques such as gun, roller or splat quenching^{4,5}. Acoustic cavitation is known to induce extreme local heating in otherwise cold liquids, and to provide very rapid cooling rates^{6–11}. Here we describe the synthesis of metallic-glass powders using the microscopically extreme (yet macroscopically mild) conditions induced by high-intensity ultrasound. The sonolysis of iron pentacarbonyl, a volatile organometallic compound, produces nearly pure amorphous iron. This amorphous iron powder is a highly active catalyst for the Fischer-Tropsch hydrogenation of carbon monoxide and for hydrogenolysis and dehydrogenation of saturated hydrocarbons.

The chemical effects of ultrasound derive primarily from hot spots formed during acoustic cavitation (that is, the formation, growth and collapse of bubbles in a liquid)^{9–11}. This process serves to concentrate dramatically the low energy density of a sound field. Our previous experiments have established^{12–14} that the effective temperature reached during bubble collapse is $\sim 5,200$ K, with a calculated hot-spot lifetime of $< 2 \mu\text{s}$. More recent sonoluminescence experiments suggest¹⁵ possible lifetimes of < 1 ns. Thus, we expect that heating and cooling rates during cavitation collapse are greater than 2×10^9 K s⁻¹ and may be as large as 10^{13} K s⁻¹.

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FIG. 1 *a*, Scanning electron micrograph of amorphous iron powder, obtained on a Hitachi S800 electron microscope. *b*, Transmission electron micrograph of amorphous iron powder, obtained on a Phillips EM 400T electron microscope.



Ultrasonic irradiation of iron pentacarbonyl, $\text{Fe}(\text{CO})_5$, yields a dull black powder. A scanning electron micrograph of the powder is shown in Fig. 1*a*. Pure $\text{Fe}(\text{CO})_5$ or 4.0 M solutions in decane were irradiated at 0 °C with a high-intensity ultrasonic probe (Sonics and Materials, model VC-600, 0.5 in. Ti horn, 20 kHz, 100 W cm^{-2}) for 3 h under argon. After irradiation, the iron powder produced was filtered and washed with dry pentane in an inert atmosphere box (Vacuum Atmospheres, <1 p.p.m. O_2). Gram quantities of material were isolated. Elemental analysis of the amorphous iron powder shows it to be >96% iron by weight, with trace amounts of carbon (3%) and oxygen (1%), presumably from the decomposition of alkane solvent or carbon monoxide during ultrasonic irradiation^{16–18}. The applications of ultrasound to chemical synthesis^{11,19–21} and useful experimental apparatus²² are described in detail elsewhere.

The amorphous nature of these powders has been confirmed by several different techniques, including scanning and transmission electron microscopy, differential scanning calorimetry, X-ray powder diffraction and electron-beam microdiffraction. Scanning electron micrographs of iron powder from ultrasonic irradiation of $\text{Fe}(\text{CO})_5$ show conchoidal fractures, typical of a non-crystalline material (Fig. 1*a*). Transmission electron microscopy show no evidence for crystallite formation to a resolution of below 4 nm (Fig. 1*b*). Differential scanning calorimetry shows one large exothermic transition at 308 °C corresponding to a disorder-order transition (crystallization) of the amorphous iron (Fig. 2).

Initial X-ray powder diffraction shows no diffraction peaks; after heat treatment under N_2 at 350 °C (sufficient to induce crystallization), the lines characteristic of α -iron metal (d spacings of 2.03, 1.43, 1.17 and 1.04 Å) are observed (Fig. 3). After crystallization, the X-ray powder diffraction pattern contains no peaks attributable to iron carbide or other iron-based phases, thus confirming the formation of essentially pure iron glass from ultrasonic irradiation of $\text{Fe}(\text{CO})_5$. This is especially noteworthy because all iron-containing metallic glasses prepared previously contain large amounts of other alloying elements (typically >20%)^{1–3,23,24}. Electron microdiffraction with a transmission electron microscope confirms these observations and shows only a diffuse ring characteristic of an amorphous material. After continued sample exposure to the electron beam and its consequent heating, the iron powder crystallizes *in situ* and the diffraction rings from α -Fe are observed.

Transmission electron micrographs reveal the microstructure of the powder (Fig. 1*b*). The large particles shown in the scan-

ning electron micrograph are composites of very small particles (~10 nm) with significant void volume. Surface areas, determined by Brunauer–Emmett–Teller gas adsorption isotherms, were found to be 120 $\text{m}^2 \text{g}^{-1}$, which is about 150 times greater than the 5- μm -diameter iron powder commercially available (Aldrich Chemicals). The sonochemically produced amorphous iron powder sinters at unusually low temperatures. At 350 °C, the amorphous powder acquires a metallic lustre and the transmission electron micrographs show loss of porosity and growth of ~50-nm crystallites. After heating, the surface area decreases by roughly one hundredfold and becomes comparable to that of the commercial crystalline powder.

We probed the catalytic activity of the amorphous iron powder for two commercially important reactions: the Fischer–Tropsch process (hydrogenation of CO) and the hydrogenolysis and dehydrogenation of saturated hydrocarbons. Catalytic studies were carried out in a continuous flow microreactor. The iron catalyst was loaded in the reaction chamber under an inert atmosphere and placed on the flow microreactor without exposure to air. The reaction products were monitored using

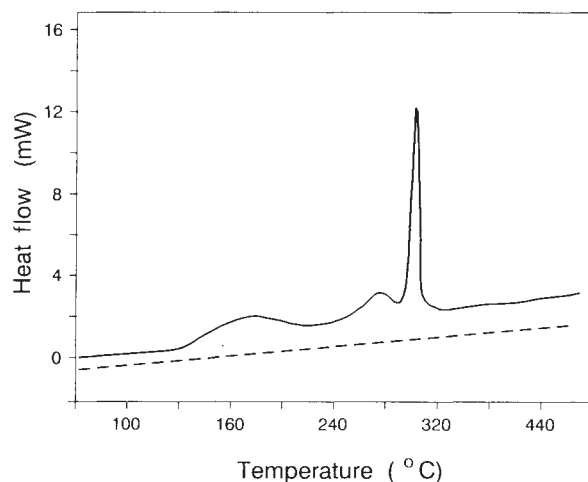


FIG. 2 Differential scanning calorimetry of amorphous (solid line) and crystalline (dashed line) iron powders, obtained at 10 °C min^{-1} on a DuPont 1090 calorimeter.

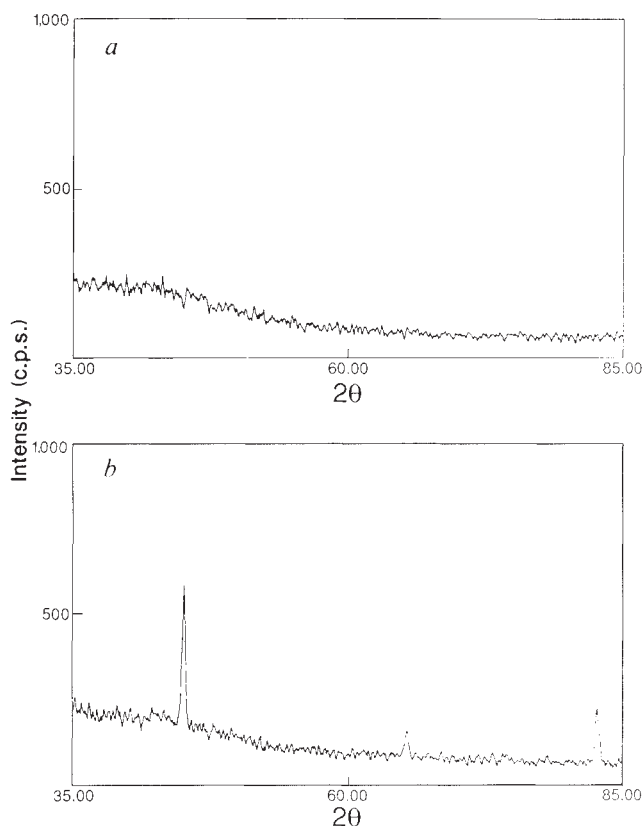


FIG. 3 X-ray diffraction powder patterns of amorphous iron powder (a) before heat treatment and (b) after crystallization at 350 °C for 6 h. Data collected on a Rigaku D-max diffractometer.

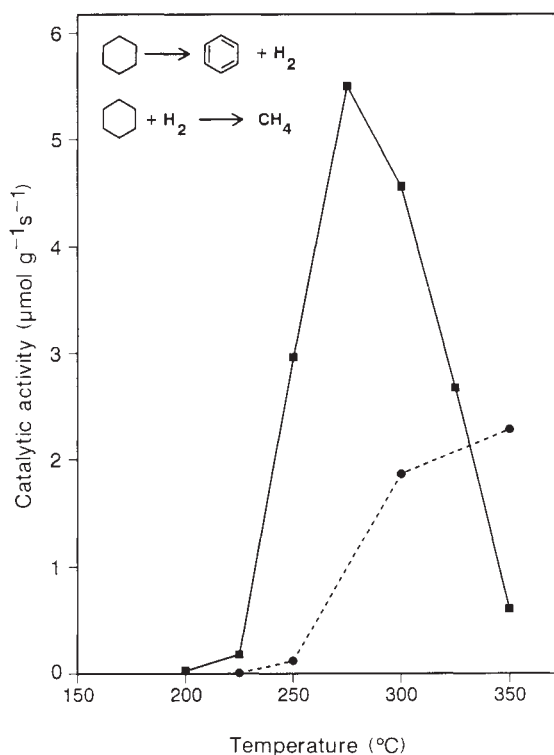


FIG. 4 The catalytic activity of amorphous and crystalline iron powder as a function of temperature for the cyclohexane dehydrogenation and hydrogenolysis reaction. Solid line, amorphous iron powder, prepared sonochemically from $\text{Fe}(\text{CO})_5$; dashed line, crystalline iron powder (5- μm diameter, Aldrich Chemicals).

gas chromatography. The Fischer-Tropsch conversion of carbon monoxide and hydrogen to low-molecular-weight alkanes occurred at very low reaction temperatures (200 °C). The amorphous powder was roughly ten times more reactive per gram than 5- μm -diameter crystalline iron powder. At 250 °C, the overall activity for cyclohexane dehydrogenation (to benzene) and hydrogenolysis (predominantly to methane) was >30 times greater for the sonochemically produced amorphous iron relative to crystalline iron (Fig. 4). We believe that the high surface area of the amorphous iron accounts for much of the increase in chemical reactivity. As expected, sintering and crystallization of the metallic glass powder at >300 °C significantly decreased its catalytic activity. The ratio of cyclohexane dehydrogenation to hydrogenolysis depended on temperature and ranged from 0.3 to 1.0.

The production of metal powder is not the only possible sonochemical reaction of $\text{Fe}(\text{CO})_5$. We have previously reported²⁵⁻²⁷ on sonochemical ligand substitution, cluster formation and catalytic alkene isomerization. The extent of CO loss can be controlled by the experimental parameters that affect cavitation collapse, including solvent vapour pressure, thermal conductivity of dissolved gas, and the ratio of heat capacities C_p/C_v of the dissolved gas. Complete ligand dissociation leading to formation of metallic glass powders occurs when the cavitation collapse is most extreme (for example, at low vapour pressure and with low gas thermal conductivity). Under such conditions, we have recently observed iron atomic emission lines in the sonoluminescence spectra (K.S.S., E. B. Flint, M.W.G. and K. A. Kemper, manuscript in preparation), confirming that iron atoms are formed during ultrasonic irradiation of $\text{Fe}(\text{CO})_5$. □

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Structure of the two-dimensional medium-pore high-silica zeolite NU-87

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SYNTHETIC aluminosilicate zeolites are widely used in the petrochemical industry as catalysts. In particular the few with multidimensional channel systems are highly valued, as they permit more complex chemistry within the pores and are less prone to deactivation through coking than are one-dimensional systems. Materials with a high silica content have improved stability towards heat in the presence or absence of steam (conditions frequently encountered in catalytic processes). NU-87 is a recently synthesized, medium-pore high-silica zeolite¹ with promising catalytic properties². Here we describe its framework topology as deduced by electron diffraction. The data were refined by comparison with X-ray powder diffraction patterns using geometric criteria, lattice-energy minimization and Rietveld refinement. The structure is unique in having a two-dimensional channel system defined by rings of 10 oxygen atoms within the framework (10-ring windows). Adjacent one-dimensional 10-ring channels are linked by short 12-ring channels to create a two-dimensional network, but access to these bridging sections is possible only via the 10-ring windows. Although the past two decades have seen considerable advances in the synthesis of novel zeolite frameworks³, NU-87 is the first high-silica 10-ring zeolite with intersecting channels to be synthesized since the industrially important ZSM-5⁴ and ZSM-11⁵ (both of which have three-dimensional channel systems) nearly 20 years ago.

Zeolites are conventionally crystalline aluminosilicates with framework structures, built by corner sharing of Si(Al)O₄ tetrahedra, enclosing cavities and channels of molecular

dimensions. These properties are exploited extensively in sorption and separation processes, ion exchange and catalysis. The last of these is arguably the most important, with zeolite-based catalysts finding widespread use in both refining and petrochemical applications. Small-pore (8-ring, typically) zeolites are useful for processing small molecules. Medium-pore (10-ring) or large-pore (≥ 12 -ring) materials are required where aromatic molecules are to be processed. Naturally occurring and early synthetic materials have framework silica/alumina ratios less than 10, but there is great interest in materials with larger values for the reasons given above. The first high-silica zeolite to be synthesized was zeolite beta⁶ (12-ring) followed closely by ZSM-5 and ZSM-11. Their channel systems were three-dimensional, but subsequent high-silica medium- or large-pore zeolites have been one-dimensional (ignoring small-pore linkages). Synthesis of zeolites of other compositions has, however, been successful, examples being the range of aluminophosphate-based frameworks³ (some novel like the 18-ring VPI-5⁷, others analogues of aluminosilicates) and the recent synthesis of the hexagonal variant of faujasite⁸ (12-ring, three-dimensional).

The sample of NU-87 used in the refinement stages of this study was prepared from a reaction mixture of composition $60\text{SiO}_2\text{-}1.5\text{Al}_2\text{O}_3\text{-}9\text{Na}_2\text{O-}2\text{NaBr-}7.5\text{DecBr}_2\text{-}3,000\text{H}_2\text{O}$, where DecBr₂ is decamethonium bromide, decane-1,10-bis(trimethylammonium bromide), the structure-directing agent or template used to facilitate the crystallization. The reaction mixture, which also contained some previously synthesized NU-87 seed crystals, was prepared using the method and reagents described elsewhere¹. The mixture was processed in a stainless steel autoclave at 170 °C, with agitation, for ~21 days. The as-made material was converted into the H-form by calcination to remove template and ion-exchange to replace Na⁺ with H⁺. The resulting material had molar composition $35\text{SiO}_2\text{-Al}_2\text{O}_3\text{-}<0.002\text{Na}_2\text{O}$. Analysis by electron microscopy revealed a rod or lath-like morphology with the majority of crystals being less than 1 μm in length.

Electron diffraction studies yielded an orthorhombic unit cell with $a = 13.7$, $b = 22.3$ and $c = 25.2$ Å and systematic absences consistent with space group $Fmmm$, $Fmm2$ or $F222$. These data indicated that NU-87 was related to nonasil⁹ and EU-1¹⁰ for

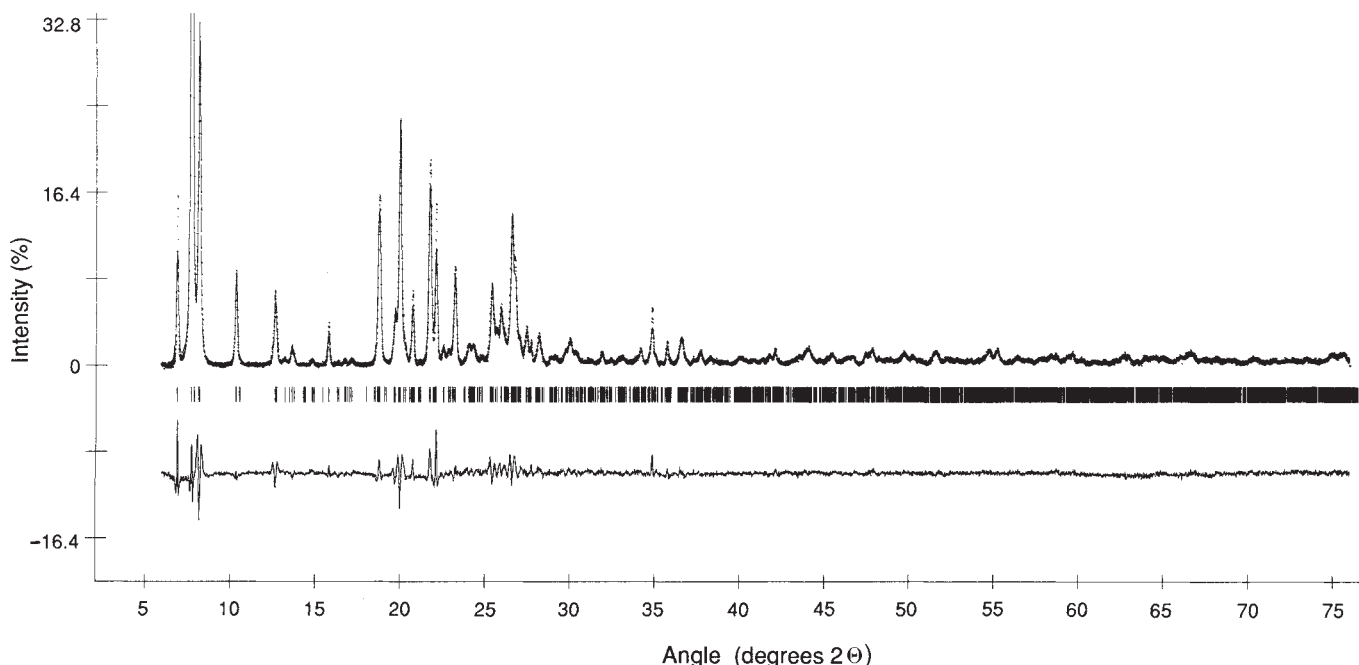


FIG. 1 Experimental (dotted) and simulated (solid) X-ray powder patterns for NU-87 (wavelength 1.50075 Å) following Rietveld refinement. Vertical

ticks indicate allowed reflections for space group $P2_1/c$. Lowest trace is difference plot.

which a and b are almost identical to those found here, while $c = 15.0$ Å for nonasil, 20.2 Å for EU-1 and 25.2 Å for NU-87. A model for the structure was proposed based on the known frameworks of these materials. The maximum topological symmetry of this model is $Fm\bar{3}m$. The model was initially refined using purely geometric constraints on bond lengths and angles (DLS-76¹¹) and the calculated powder X-ray diffraction pattern¹² was compared with experimental data. The visual agreement was sufficiently close to support the proposed topology, but minor discrepancies were noted. In addition the residual from the least-squares fit in DLS-76 was 0.017, compared with a suggested figure (C. Baerlocher, personal communication) of <0.003 for a viable structure. Further work was therefore undertaken to improve the match between the simulated and experimental powder patterns.

High-resolution studies using the powder diffraction station (9.1) at the synchrotron radiation source at Daresbury (UK) revealed additional weak reflections inconsistent with the F-centred lattice determined by electron diffraction. These have now been accounted for by a reduction in symmetry accompanied by a halving of the unit cell volume. This solution was found in an unconventional manner. The METAPOCS¹³ computer program was used to minimize the lattice energy for the topology using interaction potentials incorporating the 'shell' model for oxygen¹⁴. In this calculation every atom in the ($Fm\bar{3}m$) unit cell was treated as independent (that is, as if it were space group $P1$) although the starting coordinates were those from the DLS-76 refinement above. Examination of the resulting atom coordinates revealed that these were consistent with space group $P2_1/c$. This procedure has been found to be accurate in identifying the space group in other situations where the observed symmetry is lower than the maximum possible topological symmetry (M.D.S. and P.A.C., manuscript in preparation).

This procedure provided a good match of simulated and experimental patterns given that material in the channels was not included in the simulation. Difference Fourier syntheses indicated that there were five additional regions of electron density in the channels, and a Rietveld refinement¹⁵ of the framework plus extra-framework oxygen atoms (to model water molecules) has been carried out. Standard R -factors¹⁶ representing the quality of fit are $R_1 = 0.065$, $R_{\text{exp}} = 0.075$ and $R_{\text{wp}} = 0.14$. The unit cell contains 68 Si or Al and 136 O atoms and its refined dimensions are $a = 14.324(2)$ Å, $b = 22.376(1)$ Å, $c = 25.092(4)$ Å and $\beta = 151.515(5)^\circ$. (Note that a in this cell corresponds to $1/2$ $[101]$ in the $Fm\bar{3}m$ cell).

Some lines in the X-ray powder pattern are significantly sharper than others. In zeolites this feature is frequently due to planar faulting but can also be due to rod or platelet morphology. In this case there is no evidence for planar faulting in our electron microscopy results. Most of the remaining discrepancies in intensity between the Rietveld calculation and experiment (Fig. 1) can be accounted for by preferential line-broadening as a result of the lath-like crystal morphology of this sample. The Rietveld method does not account for this effect. Any preferred orientation effects on relative intensities that might have arisen from this morphology should have been eliminated by the spinning capillary used to contain the sample. The reason for the clear discrepancy between our electron and X-ray diffraction space groups is being investigated further. A number of factors may be involved (dehydration in vacuo, beam heating or beam damage), leading to a phase change in the electron microscope.

The framework topology of NU-87 is shown in Fig. 2 and fractional atomic coordinates from the Rietveld refinement are listed in Table 1. The Si-O bond lengths have a mean of 1.60 Å and range from 1.57 to 1.64 Å, while the O-Si-O bond angle range is closely grouped around the tetrahedral angle between

TABLE 1 Fractional atom coordinates for $P2_1/c$ asymmetric unit of NU-87 from Rietveld refinement

Atom	x	y	z	Atom	x	y	z
T(1)	0.640 (2)	0.1717 (6)	0.324 (1)	O(12)	0.350 (3)	0.0523 (8)	0.269 (2)
T(2)	0.473 (2)	0.1970 (5)	0.738 (1)	O(13)	0.318 (4)	0.0626 (8)	0.066 (2)
T(3)	0.028 (2)	0.2218 (5)	0.500 (1)	O(14)	0.675 (3)	0.0611 (7)	0.757 (2)
T(4)	0.228 (2)	0.0010 (6)	0.178 (1)	O(15)	0.651 (3)	0.0530 (7)	0.911 (2)
T(5)	0.226 (2)	0.0039 (6)	0.048 (1)	O(16)	0.240 (2)	0.163 (1)	0.206 (2)
T(6)	0.412 (2)	0.1280 (6)	0.108 (1)	O(17)	0.244 (3)	0.1761 (9)	0.023 (1)
T(7)	0.421 (2)	0.1200 (7)	0.308 (1)	O(18)	0.880 (3)	0.152 (1)	0.833 (2)
T(8)	0.666 (2)	0.1299 (6)	0.737 (1)	O(19)	0.873 (3)	0.146 (1)	1.031 (2)
T(9)	0.654 (2)	0.1237 (6)	0.924 (1)	O(20)	0.508 (4)	0.133 (1)	0.409 (2)
T(10)	0.606 (2)	0.1816 (7)	0.496 (1)	O(21)	0.535 (3)	0.134 (1)	0.116 (2)
T(11)	0.604 (2)	0.1838 (7)	0.109 (1)	O(22)	0.533 (3)	0.137 (1)	0.619 (2)
T(12)	0.456 (2)	0.1822 (7)	0.536 (1)	O(23)	0.563 (4)	0.138 (1)	0.935 (2)
T(13)	0.451 (2)	0.1837 (7)	0.917 (1)	O(24)	0.521 (5)	0.2479 (9)	0.440 (2)
T(14)	0.035 (2)	0.1941 (6)	0.100 (1)	O(25)	0.526 (5)	0.2499 (8)	0.587 (2)
T(15)	0.038 (2)	0.2969 (6)	0.406 (1)	O(26)	0.555 (4)	0.163 (1)	0.531 (2)
T(16)	0.033 (2)	0.2154 (6)	0.307 (1)	O(27)	0.509 (4)	0.167 (1)	0.006 (2)
T(17)	0.031 (2)	0.2175 (6)	0.697 (1)	O(28)	0.838 (3)	0.178 (1)	0.605 (2)
O(1)	0.242 (3)	0.009 (1)	0.121 (2)	O(29)	0.837 (2)	0.180 (1)	0.221 (2)
O(2)	0.550 (4)	0.2367 (7)	0.284 (2)	O(30)	0.223 (2)	0.176 (1)	0.414 (2)
O(3)	0.870 (2)	0.1734 (8)	0.434 (2)	O(31)	0.219 (3)	0.176 (1)	0.793 (2)
O(4)	0.242 (2)	0.194 (1)	0.614 (2)	O(32)	0.026 (5)	0.2544 (8)	0.640 (2)
O(5)	0.588 (3)	0.133 (1)	0.349 (2)	O(33)	0.037 (5)	0.2734 (8)	0.345 (2)
O(6)	0.553 (3)	0.141 (1)	0.228 (1)	O(34)	0.036 (5)	0.2360 (9)	0.248 (2)
O(7)	0.579 (4)	0.168 (1)	0.741 (2)	X(1)	0.233 (8)	0.037 (3)	0.568 (5)
O(8)	0.533 (3)	0.159 (1)	0.818 (2)	X(2)	0.39 (1)	0.016 (6)	0.917 (5)
O(9)	0.002 (4)	0.2773 (8)	0.526 (2)	X(3)	0.08 (1)	0.044 (3)	0.268 (5)
O(10)	0.006 (4)	0.2429 (8)	0.423 (2)	X(4)	-0.03 (1)	0.037 (2)	0.313 (6)
O(11)	0.001 (2)	0.003 (1)	0.083 (1)	X(5)	0.01 (2)	0.065 (4)	0.61 (1)

Numbers in parentheses are the estimated standard deviation in units of the least significant figure. X represent extra-framework scatterers, probably water, modelled by oxygen.

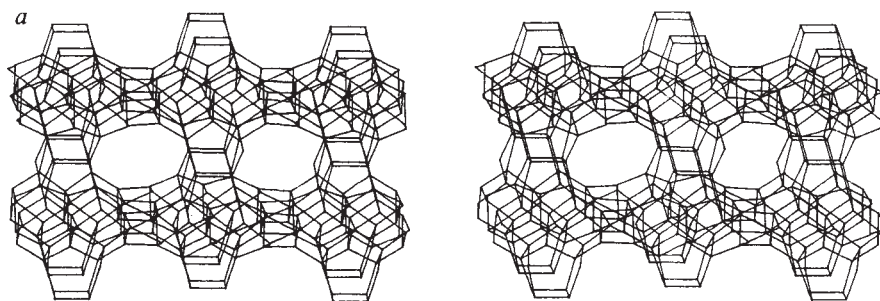
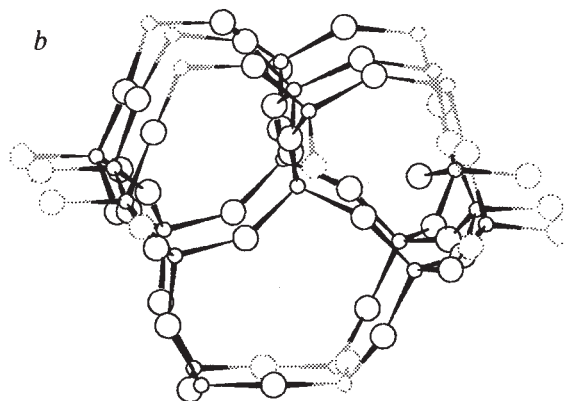


FIG. 2 NU-87 framework topology. *a*, Stereo view along [201] axis in $P2_1/c$ cell with Si or Al atoms at vertices (O atoms between these omitted for clarity) and *b*, ball-and-stick model at right angles to *a* (larger circles are oxygen, solid atoms belong to asymmetric unit).



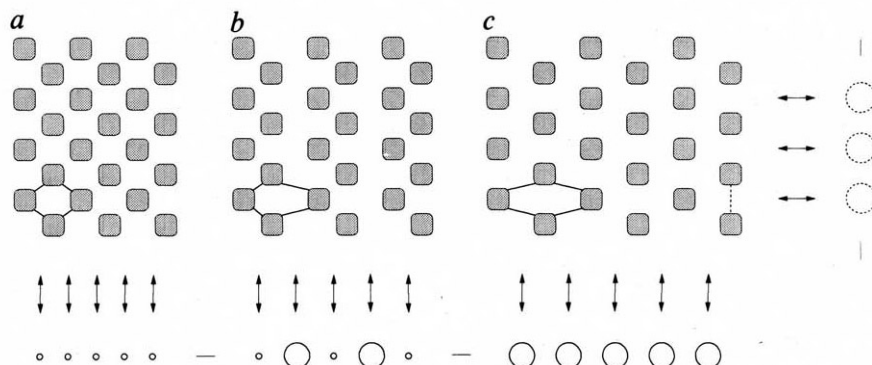
107° and 113°. These were subject to soft constraints in the refinement.

The framework topology of NU-87 is simply related to those of nonasil and EU-1. Each of these can be considered to have impermeable sheets held apart by pillars: 4-rings inserted between and parallel to the sheets. These can be clearly seen in the horizontal mid-plane of Fig. 2*a*. The separation of the 4-rings in the plane between the sheets (Fig. 3) determines the size of the windows between the pillars. Nonasil contains effectively closed cages, as the maximum ring size between the pillars is 6. Within a cage these rings occur in pairs, with a pair at each end of the cage, and link each cage to its four nearest neighbours (Fig. 3*a*). In EU-1 the pair of 6-rings at one end of the cage 'open up' to 10-rings and produce straight, one-dimensional 10-ring channels, but with the unique feature of deep 12-ring side-pockets (Fig. 3*b*). In NU-87 the remaining 6-rings from EU-1 (at the bottom of the side-pockets) 'open up' to produce straight 10-ring channels, parallel to the first set, but now with 12-ring bridges between adjacent 10-ring channels. Because

these bridges are on alternating sides along the length of the 10-ring channels, all access to these 12-ring bridges is through 10-ring windows (Fig. 3*c*). Based on the Rietveld refinement, the 10-ring channel dimensions of NU-87 normal to [201] are alternatively 4.6 Å × 6.2 Å and 4.8 Å × 5.9 Å along each channel, and the 12-ring linkage is approximately 5.3 Å × 6.8 Å. Thus the limiting 10-rings have a similar projected area to those in ZSM-5 and ZSM-11, but are less circular.

The only synthetic aluminosilicate zeolites other than ZSM-5 and ZSM-11 that had previously met the criterion of multi-dimensional, ≥10-ring channels were beta⁴ and the relatively low-silica faujasites (Linde X, Y^{17,18} and the new hexagonal variant⁸), all of which are three-dimensional 12-ring systems. Given that rarity, it is easy to understand the current great interest¹⁹ in trying to synthesize high-silica analogues of the recently discovered mineral boggsite²⁰, which has 10- and 12-ring windows bounding a three-dimensional channel system. NU-87 is clearly an interesting addition to the zeolite family. □

FIG. 3 Schematic plan views along [010] and partial elevations of channel systems in *a*, nonasil, *b*, EU-1 and *c*, NU-87. Rounded squares represent 4-rings at centre of pillars between impermeable silicate sheets. Solid lines on plans indicate the position of the 6-ring (small) and 10-ring (large) windows shown in elevation (solid circles) beneath each plan: thus in *a*, the four lines represent four 6-ring windows; in *b* they represent two 6-ring and two 10-ring windows, and so on. Dashed lines and circles indicate 12-ring bridges.



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Do upper-ocean sediment traps provide an accurate record of particle flux?

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SEDIMENT traps are widely used to measure the vertical flux of particulate matter in the oceans. In the upper ocean, sediment traps have been used to determine the extent to which CO_2 fixed by primary producers is exported as particulate organic carbon^{1–3}. In addition, the observed decrease of particle flux with depth has been used to predict regeneration rates of organic matter and associated elements³. Over seasonal or annual timescales, the import of limiting nutrients into the upper ocean (new production) should be balanced by particle export^{4,5}. Given the importance of accurately determining the sinking particle flux, it has been suggested that ^{234}Th might be used to 'calibrate' shallow-trap fluxes⁶. Here I present a re-evaluation of existing ^{234}Th data which indicates that trap-derived and model-derived ^{234}Th particle fluxes can differ by a factor of ± 3 –10, suggesting that shallow traps may not provide an accurate measure of particle fluxes.

The activity of ^{234}Th (half-life 24.1 days) in the oceans is primarily controlled by production from its soluble parent, ^{238}U (half-life 4.5×10^9 years), and losses through radioactive decay plus sorptive removal on sinking particles. A typical ^{234}Th profile in the open ocean shows relatively low ^{234}Th activities in surface waters and an increase with depth as the scavenging intensity decreases (Fig. 1). As ^{238}U is conservative in sea water, the activity of ^{238}U varies little with depth (Fig. 1) and is proportional to salinity (^{238}U (d.p.m. l^{-1}) = $0.069 \times \text{salinity}$ ⁷). Secular equilibrium between ^{238}U and ^{234}Th usually occurs at depths of 50–200 m.

The deficiency of total ^{234}Th relative to ^{238}U has been widely used as a measure of the uptake and removal of ^{234}Th through particle scavenging^{8–14}. The magnitude of ^{234}Th export from the surface ocean on sinking particles can be calculated from the following equation^{8–11}

$$\partial^{234}\text{Th}/\partial t = ^{238}\text{U} \times \lambda - ^{234}\text{Th} \times \lambda - P \quad (1)$$

where ^{234}Th is the measured activity of total ^{234}Th , ^{238}U is determined from salinity, $\partial^{234}\text{Th}/\partial t$ is the change in ^{234}Th activity

with time, λ is the decay constant for ^{234}Th (0.0288 day^{-1}) and P is the net removal flux of ^{234}Th by particles. This equation can be solved for steady-state ($\partial^{234}\text{Th}/\partial t = 0$) and non-steady-state conditions, and assumes that advection and diffusion terms can be neglected (see discussion). Using equation (1), it is possible to predict particle export at a given depth for ^{234}Th by integrating total ^{234}Th and ^{238}U activities from the surface to the depth of interest^{12,15} and calculating the unknown flux term P . The particle flux term will reflect the net sum of all biotic and abiotic particle formation, exchange, remineralization and export processes. This simple vertical ^{234}Th scavenging model forms the basis for the calibration of sediment trap fluxes.

Figure 2 summarizes the results from a variety of open ocean, coastal, and semi-enclosed basin sites where it is possible to compare the measured trap flux of ^{234}Th with the predicted particle ^{234}Th flux calculated from water-column data using equation (1). All of the studies shown employed shallow sediment traps which are similar or identical to the VERTEX cylindrical design¹⁶. This is by far the most common trap design used in the upper oceans in the last decade, and few results for ^{234}Th exist from studies using any other type of trap. The model ^{234}Th flux data were either taken as reported from the original source, or calculated by this author (see Table 1 for details). The data are plotted as the logarithm of the ratio of the sediment trap ^{234}Th flux to the model-derived ^{234}Th flux. Relative to the model fluxes, data above $\log 0 (=1)$ suggest a positive collection bias and data below this line a negative collection bias. Roughly one third of the data suggests collection biases of at least a factor of three, and two thirds of the points lie at or beyond $\pm 50\%$ (Fig. 2).

The extent to which the trap and model fluxes agree or disagree seems to be independent of the trap depth, total flux or research laboratory where the ^{234}Th analyses were made (Table 1). Within a given site one tends to find consistently either under- or over-collection biases which are much larger than the error of any individual ratio. Given these data, one must conclude either that (1) shallow-trap particle fluxes can be substantially biased in either a positive or negative direction, and/or that (2) the simple ^{234}Th scavenging model (equation (1)) is not appropriate for calibrating shallow-trap fluxes. I will re-examine the ^{234}Th scavenging model first, as I propose that it is more likely that the trap fluxes are in error than the model-derived fluxes.

In most of the studies represented in Fig. 2, single profiles of ^{234}Th and the assumption of steady state were used to calculate the ^{234}Th particle flux (Table 1). The magnitude of the error introduced by assuming steady state ($\partial^{234}\text{Th}/\partial t = 0$ in equation (1)) will depend upon the specific setting. For example, in the

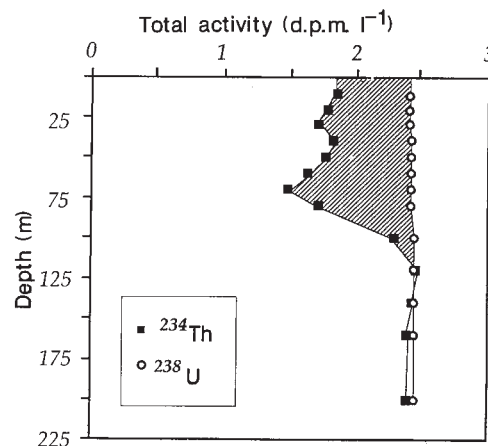


FIG. 1 Typical profile of ^{234}Th in the upper open ocean. Shaded area represents disequilibrium between total ^{234}Th and ^{238}U . Data taken from VERTEX 3 (ref. 11).

TABLE 1 Compilation of ^{234}Th flux data from traps and model

Identification number	Ref. number	^{234}Th flux		Res.-t (days)	Trap-t (days)	Depth (m)	Station ID	Model type	Location (and comments)
		Calc. (d.p.m. $\text{m}^{-2} \text{d}^{-1}$)	Trap (d.p.m. $\text{m}^{-2} \text{d}^{-1}$)						
1	8	1,500	800	19	13	50	VERTEX 1	SS	Different stations within California Current
2		1,500	2,100	19	13	50	VERTEX 1		
3		1,300	900	21	6	50	Cerop I		
4		1,700	2,000	18	5	50	Cerop II		
5		1,700	1,300	21	8	65	Cerop III		
6		1,800	2,200	20	6	60	MLML-2		
7	12	800	1,020	6	14	10	3/1-15/82	NSS	Funaka Bay, Japan (March data provided best temporal comparison, other months show same range)
8		3,000	870	18	14	40	3/1-15/82		
9		5,000	2,390	16	14	80	3/1-15/82		
10	11	570	835	26	22	30	VERTEX 2	SS	Off Manzanillo, Mexico
11		1,222	1,878	30	22	120	VERTEX 2		
12		1,600	1,505	25	21	80	VERTEX 3		
13		1,861	730	27	21	120	VERTEX 3		
14	13 & 32	630	671	33	34	150	VERTEX 4	SS	900 kmN of Hawaii
15		1,660	175	31	10	200	Feb. 87		
16		604	148	33	10	200	Mar. 88		
17		316	181	34	15	200	Apr. 87		
18	14	744	1,960	30	3	80	sta-1	SS	Panama Basin (calculated fluxes from equation (1) by this author)
19		620	2,060	31	3	80	sta-2		
20		1,084	1,600	29	3	80	sta-5		
21	19	813	693	10	3	20	Feb.	SS	Dabob Bay, Washington, U.S.A. (calculated fluxes from equation (1) by this author, and data below 50 m are excluded because of evidence of sediment resuspension)
22		1,850	1,181	13	3	50			
23		797	365	13	3	19	March		
24		1,471	352	13	3	39			
25		1,133	168	8	3	25	May		
26		1,960	290	9	3	45			
27		969	635	8	3	23	June		
28		1,719	977	10	3	43			
29		942	722	9	3	23	July		
30		1,554	1,238	11	3	43			
31		869	168	8	3	20	Aug.		
32		1,575	970	10	3	40			
33		1,001	980	9	3	24	Sept.		
34		1,688	999	11	3	44			
35		969	632	10	3	25	Oct.		
36		1,708	1,160	11	3	45			
37		1,111	358	9	3	28	Nov.		
38		1,850	683	8	3	48			
39	20	253	380	29	3	36	BS3 sta-2	SS	Black Sea (calculated fluxes from equation (1) by this author)
40		480	822	29	3	71			
41		78	354	33	3	40	BS3 sta-6		
42		6.3	520	35	3	75			
43	15	4,565	1,867	25	11	150	late April	NSS	Northeast Atlantic
44		4,257	1,279	29	11	300			
45		3,093	2,145	27	14	150	early May		
46		7,745	1,485	28	14	300			
47		5,061	1,571	23	11	150	end May		
48		3,582	1,498	29	11	300			
49	18	949	4,537	25	13	50	VERTEX 5	SS	California Current (calculated fluxes from equation (1) by this author)
50		1,158	4,574	29	13	100			
51		1,900	692	25	13	100	VERTEX T7		Off Alaska (station Papa)

Identification numbers refer to Fig. 2. Calculated ^{234}Th flux based on equation (1); trap ^{234}Th flux from original reference. Res.-t is the residence time of ^{234}Th with respect to decay and particle removal (see text). Trap-t is the trap deployment duration. Station ID provided as in original reference. Model type: SS, steady state; NSS, non-steady state.

Pacific gyre, seasonal variations in the ^{234}Th inventory in the upper ocean are expected to be small and thus assumption of steady state is reasonable^{17,18}. In contrast, at the onset of the spring bloom in the North Atlantic, the calculated particle flux can vary by as much as a factor of four if the change in ^{234}Th activity with time is ignored¹⁵. Figure 2 includes studies where steady-state and non-steady-state solutions to equation (1) have been considered, and in both cases agreement with the trap fluxes is poor.

Neglected in equation (1) are vertical advective and diffusive fluxes of ^{234}Th . Under average oceanic conditions these factors are small relative to the particle flux (for example, 5–10% of the particle flux¹⁵). An important point is that vertical mixing would always tend to supply ^{234}Th to the surface layer given the increased ^{234}Th activities at depth (Fig. 1). Ignoring this supply would cause the calculated fluxes from equation (1) to be too small. This would produce an overall systematic positive collection bias which is not seen in the data (Fig. 2).

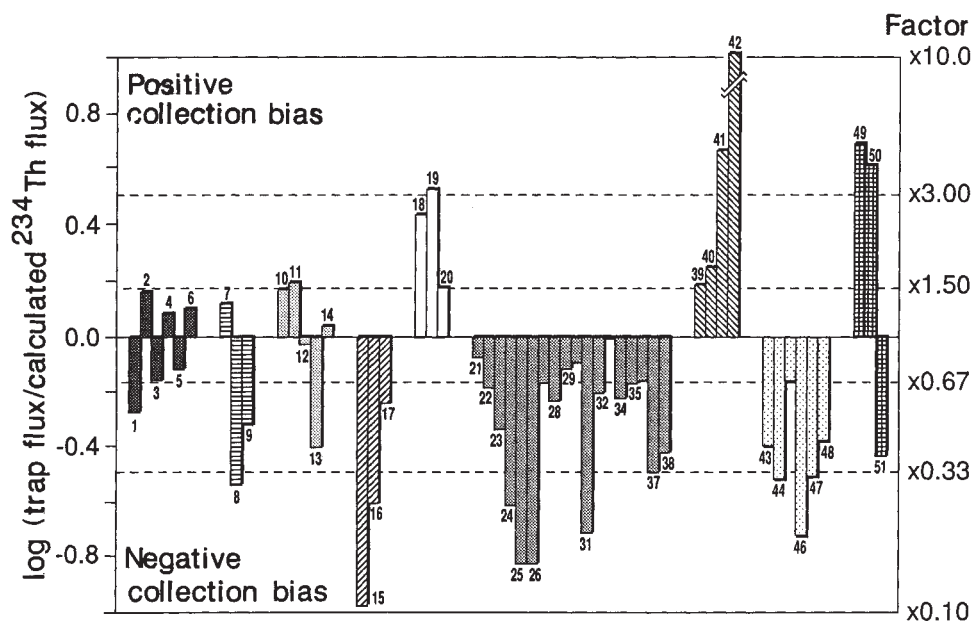


FIG. 2 Bar diagram showing the comparison between ^{234}Th fluxes measured in shallow sediment traps, and that calculated for the same depth and time using the ^{234}Th scavenging model (equation (1) in text). Data are plotted as the logarithm of the ratio of the trap flux to the model-derived ^{234}Th flux. Each bar is numbered for reference to Table 1 and different fill patterns are used for each study. The left-hand vertical scale shows the ratio as the logarithm, and the right-hand scale converts this ratio to the factor of positive or negative collection bias suggested by the data (see text for details).

Finally, one must consider whether or not ^{234}Th fluxes measured in traps and those determined from the water column ^{234}Th distributions reflect particle export over the same scales. If the traps are deployed for only a few days (see Table 1) episodic particle fluxes may be missed, or alternatively short-term periods of low flux may not be represented. This factor alone should produce a random bias of over- and under-collection at a given site. It can be seen, however, that in traps deployed for only a few days, either in one season or throughout the year^{14,19,20}, the calibration is not random and is biased in a single direction (Table 1).

If steady state is assumed, the deficiency in total ^{234}Th reflects a temporal scale determined by the residence time of ^{234}Th with respect to radioactive decay and scavenging ($t = 1/(\lambda + k)$, where k is the particle removal rate). This residence time ranges from 8 to 35 days for the studies compiled here (Table 1). In many cases this is comparable to the duration of the trap experiment. In the studies that applied a non-steady-state solution to equation (1) ^{234}Th profiles are obtained at trap deployment and retrieval times and thus $\partial^{234}\text{Th}/\partial t$ is measured to calculate the particle flux during the trap deployment^{12,15}. In this case the temporal scale for the trap and model fluxes is the same. In general, the non-steady-state approach is preferred. The two non-steady-state studies referenced here suggest a negative collection bias for traps of up to a factor of three (Table 1).

There is still some uncertainty over whether the spatial scales of particle export over which the traps and ^{234}Th measurements integrate are comparable. Patchiness in particle flux measured by traps would not, however, be expected to produce the unidirectional biases seen in the calibration at a given site (Table 1). Multiple traps and ^{234}Th measurements over spatial scales of 10–100 km would be needed to answer this question directly.

It seems that the ^{234}Th balance and hence particle export flux in the ^{234}Th scavenging model is well constrained. The disagreement between the calculated and measured particle fluxes has not been eliminated or even reduced when more rigorous non-steady-state models have been used. Limitations in the model would tend to produce random biases in the flux estimates, but unidirectional trends are most often found within a given site. If the errors in the model are small, or at least random, then one must consider why the traps might over- or under-collect the true ^{234}Th particle flux at a given site. The potential problems associated with using a shallow sediment trap as a collector of particle flux can be broadly divided into two areas, namely sample integrity and hydrodynamics.

Sample integrity issues will be of primary concern for carbon and organic constituents rather than for ^{234}Th . For example, it has long been recognized that some organisms, the so-called swimmers^{6,21}, will actively enter shallow traps. For ^{234}Th , swimmers pose much less concern, as the specific activity of ^{234}Th in potential swimmer fractions (salps and zooplankton) is lower by 1–3 orders of magnitude than that found in trap material²². Sediment samples remain in the trap for days or weeks depending on the duration of trap deployment. Consequently the loss of carbon and nutrients to the trap solution phases can occur²³. As ^{234}Th is strongly adsorbed on particle surfaces, sample integrity is ensured⁶.

Sediment traps of any kind will alter the flow field around them and thereby produce a hydrodynamic bias for collecting settling particulate matter. This would affect both the observed ^{234}Th and organic matter fluxes. Considerable effort has been spent in evaluating various trap designs, and the VERTEX-style cylinders used in the ^{234}Th fluxes referenced here were identified in early studies as having a minimal particle collection bias^{24,25}. For a given design, however, it is known that variations in the magnitude of horizontal currents will affect the flow field thus potentially biasing the overall collection efficiency^{26–28}. Because multiple traps are typically hung from a single free-floating mooring, shallow traps in the field move at different speeds relative to the water in a complicated depth- (and time-) dependent pattern. Tilt and wave effects may also produce additional hydrodynamic biases for floating traps²⁹.

In the oceans, a wide range of particle types has been found, with sinking speeds ranging from <1 m per day to >1,000 m per day³⁰. Recent laboratory experiments²⁷ indicate that the bias due to fluid dynamics varies for different particle types under different horizontal flows. It can be postulated that the disagreement between trap and model-derived fluxes seen in Fig. 2 is due to differences in flow and particle type at a given location. Additional factors may, however, be significant. For example, vertical migrating organisms may actively remove particles from the upper ocean and bypass the traps during their descent to depth³¹. This would result in an apparent under-collection by the traps. It should also be noted that a calibration of particle flux using ^{234}Th may not hold for organic carbon or other elements if the particle classes that carry these elements differ. On the other hand, if the traps cannot be shown to collect quantitatively the particles that carry ^{234}Th , then there is good reason to question the accuracy of other flux measurements.

Sediment traps have contributed considerably to our understanding of sinking particles in the oceans. They provide samples for analyses, as well as what is thought to be reasonable flux information. A quantitative evaluation of trapping efficiency is needed, however, and the calibration of sediment trap fluxes using ^{234}Th provides a powerful method of doing this. At present, the ^{234}Th water column measurements and scavenging models suggest that over- or under-collection by a factor of three or more is common in shallow traps. A predicted flux within this uncertainty may be adequate for many purposes. But this calibration does not reflect artefacts due to swimmers and sample integrity, which will result in an additional collection bias for

organic constituents. For the calibration to hold, we must continue to examine the assumptions in the ^{234}Th scavenging model. More time-series data and a three-dimensional grid of ^{234}Th profiles and sediment traps will be needed to examine the spatial scale of particle export and to quantify any horizontal fluxes which are ignored in the simple vertical scavenging models. We may not yet be able to give a definitive answer to the question posed in the title. The disagreement between particle ^{234}Th fluxes measured by traps and that predicted from water column data suggests, however, that the answer is not likely to be in the affirmative. □

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Erosion of a stable density gradient by sedimentation-driven convection

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STABLE density gradients are common in the world's oceans and lakes, and play a crucial part in their physical, chemical and biological evolution. It has recently been suggested¹ that turbidity currents provide an indirect mechanism for eroding this stratification. In these currents, light ambient fluid that has been mixed by cyclones with dense suspended sediment is transported to greater depths, where it convectively erodes the gradient as the sediment settles. Here I present experimental results which show that in these circumstances the density gradient is eroded in a number of cycles of decreasing intensity and increasing duration. In each cycle some of the sediment settles to the base of a stagnant region of unimpeded sedimentation, while the rest is mixed into an overlying region that is vigorously convecting. My experimental observations agree with a simple physical model that predicts the rate and extent of vertical mixing as well as the variation of particle sizes in the resulting turbidite.

The experiments were carried out in a Perspex tank ($30 \times 15 \text{ cm}^2$ in area, 65 cm deep). The double-bucket technique^{2,3} was used to fill the tank with a sugar solution, the density of which increased linearly with depth from $\rho_1 = 0.999 \text{ g cm}^{-3}$ to 1.020 g cm^{-3} . The experiments were then initiated by carefully adding a dense suspension at the bottom of the tank using a pipe leading from a continuously stirred reservoir. The suspension had a bulk density $\rho_B = 1.034 \text{ g cm}^{-3}$, and was composed of dyed fresh water (density ρ_1) and spherical glass beads (density 2.47 g cm^{-3} ; median diameter $23.5 \mu\text{m}$ with a standard deviation $\sim 6 \mu\text{m}$).

The evolution of the experiments is shown schematically in Fig. 1, commencing in Fig. 1a with the initial condition of a stratified layer underlain by a suspension. Observations using a microscope revealed that the initial motions generated during the input of the suspension were rapidly dissipated. The glass beads subsequently settled at a range of velocities that reflected their range in size, and a layer of sediment accumulated on the base of the tank. At the top of the suspension, sedimentation created a buoyant layer of interstitial fluid and some suspended sediment, which rose in tendrils to drive vigorous convection in an overlying region (Fig. 1b). The input of these buoyant tendrils from below continuously decreased the bulk density of the well-mixed convecting region, and in turn led to the steady erosion of the overlying density gradient. In contrast to the sharp interface at the base of the convecting region, the interface with the density gradient was diffuse (Fig. 2).

The convecting region continued to grow at the expense of both the underlying sedimenting region and overlying gradient (Fig. 1c) until the sharp interface had reached the base of the tank. At this time, the source of buoyancy for the convecting region was lost, and the convective motions rapidly dissipated, leaving a situation (Fig. 1d) similar to the initial condition (Fig. 1a): a well-mixed suspension underlying a gradient. Hence it was not surprising that a new sharp interface developed as part of another cycle of coupled sedimentation and convection (Fig. 1e). This second cycle was slower, the convection was weaker and the erosion of the gradient less. By the end of the second cycle, the concentration of suspended sediment was very small, so that, although further cycles seem plausible, they could not be discerned. After a long time, all the sediment had settled, convection had ceased, and the fluid region consisted of a homogeneous region overlain by the remaining gradient (Fig. 1f).

A simple physical model of this phenomenon has been developed based on the experimental observations. Consider first the behaviour of the stagnant sedimenting region. In this region, the particles settle at a range of velocities, which leads to the suspension becoming smoothly stratified in its bulk

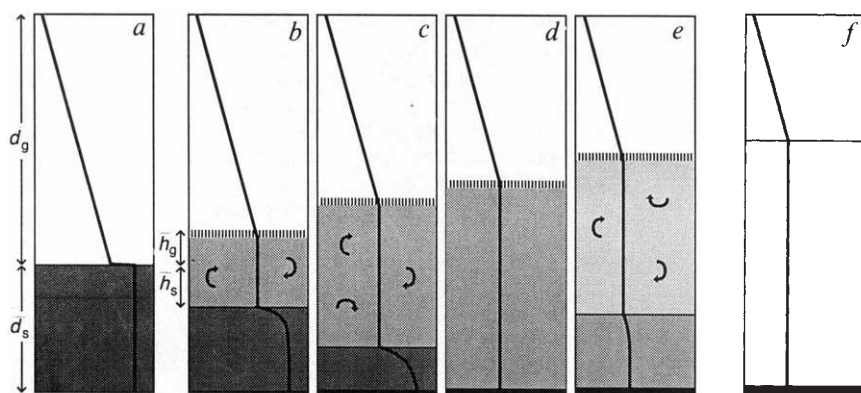


FIG. 1 Schematic sketch of the cycles of coupled sedimentation and convection, and the corresponding changes in the bulk-density profile, that occur when a suspension is emplaced underneath a density gradient. Also indicated are the initial depths of the suspension ($d_s = 20$ cm) and of the gradient ($d_g = 40$ cm), and the extent of erosion of the gradient (h_g) for a given fall (h_s) of the top of the suspension.

density, with only interstitial fluid of density ρ_l at the top, fluid plus the smaller particles lower down, and with all the particle sizes and the initial bulk density of ρ_B at greater depths. The shape of this bulk-density profile is easily calculated⁴, and is given simply, if hindered settling⁵ is ignored, by the cumulative distribution $v(F)$ of the fraction F of the particles with settling velocities less than v (Fig. 3). Throughout the experiments, the evolving stratification within the sedimenting suspension is overlain by fluid with a density ρ_U that is intermediate between ρ_l and ρ_B , so that the upper parts of the suspension that have bulk densities less than ρ_U are buoyant and can convect away. Hence the minimum velocity of the sharp interface is given by the descent velocity $v_{\min}$ of the neutrally buoyant part of the suspension that contains a fraction of the initial particles equal to the density ratio $R = (\rho_U - \rho_l)/(\rho_B - \rho_l)$:

$$v_{\min} = v(R) \quad (1)$$

Both $v_{\min}(R)$ and the corresponding fraction $X_{\min}(R)$ of sediment lifted (see Fig. 3) are underestimates because the buoyant part of the suspension have the potential to entrain some of the underlying negatively buoyant parts as they convect away. But recent measurements (R.C.K. and J.R. Lister, manuscript in preparation) have shown that the efficiency of this entrainment is small, so that the theoretical lower bounds $v_{\min}$ and $X_{\min}$ are good estimates of the observed values.

During the first cycle of sedimentation and convection, the density ratio is related to the height $h_g(t)$ by which the protrusion interface has risen

$$R(t) = R_0[1 - h_g(t)/d_g] \quad (2)$$

where R_0 is the initial density ratio and d_g is the initial depth of the density gradient. The value of $R(t)$ determines the instantaneous values of $v_{\min}(R(t))$ and $X_{\min}(R(t))$. Hence the height

h_s that the sharp interface has fallen is given by

$$\frac{dh_s}{dt} = v_{\min}(R(t)) \quad (3)$$

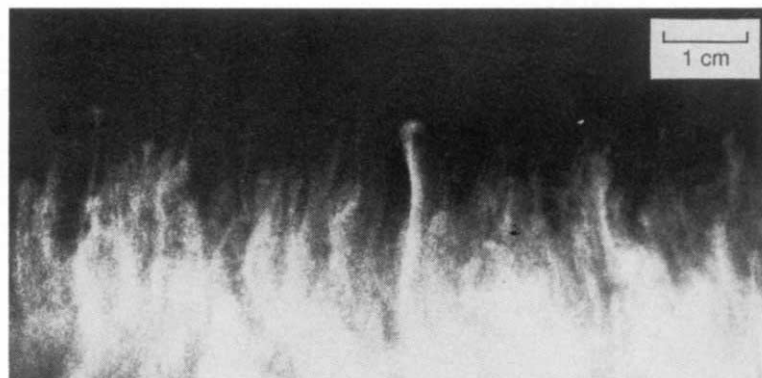
while conservation of mass relates h_s and h_g by

$$\frac{dh_g}{dh_s} = \frac{d_g[1 - X_{\min}(R(t))/R_0] - h_g}{h_s + h_g} \quad (4)$$

Given the functions $v_{\min}(R)$ and $X_{\min}(R)$, the system of equations 2-4 can be integrated numerically to determine the evolution of h_s and h_g . The calculated position of the sharp interface with time is shown with the experimental observations in Fig. 4a. The agreement at early times is excellent, although at later times the interface falls at a velocity somewhat greater than $v_{\min}(R)$. This systematic departure is due to the steady increase in the depth of the convecting region, which increases both the vigour of the convection and the entrainment efficiency (J. C. K. and Lister, manuscript in preparation). Figure 4b presents the observations of h_s and h_g , together with the theoretical curve predicted by equations (2-4). The agreement is excellent.

In addition to the results shown in Fig. 4, the model applied here can be used to predict the rate of accumulation of sediment on the floor and the particle size distribution as a function of height in this sediment. The model also predicts the concentration and size distribution of the particles in the convecting region, and hence can predict the evolution of the second (Fig. 1e) and subsequent convective cycles. The completion of each of these cycles corresponds to a discontinuity in the size distribution in the final settled sediment. The convection and gradient erosion in the later cycles is much weaker because of the small particle sizes, large suspension depths and low sediment concentrations that remain at the end of the first cycle.

FIG. 2 Photograph of the buoyant, particle-laden protrusions that penetrate the stable stratification at the top of the convecting layer. The photograph shows the light scattered by individual particles when the tank is illuminated from the side at right angles to the line of sight. The protrusions contrast markedly with observations of 'domes', 'cusps', 'folds' and 'waves' in the entrainment zone when a stable thermal gradient is heated from below^{17,18}. The protrusions look similar to double-diffusive 'salt fingers'^{19,20}. The physics of their formation is fundamentally different, however, because it does not involve diffusion. It is suggested that the protrusions develop from a local gravitational instability at the top of the convecting region. The instability arises because the vertical turbulent convective velocities decrease towards the top of the convecting region, thus allowing sedimentation to decrease the local bulk density.



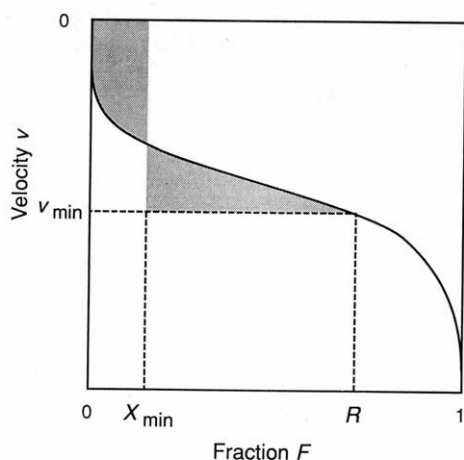


FIG. 3 Schematic sketch of a typical cumulative distribution of the fraction F of particles settling at speeds less than velocity v . If hindered settling⁵ is ignored, the $v(F)$ curve indicates the bulk density ρ of a suspension at a depth d after sedimentation for a time t , plotted in the form of d/t against $(\rho - \rho_l)/(\rho_B - \rho_l)$, where ρ_l and ρ_B are, respectively, the interstitial density and initial bulk density. When overlain by fluid with density ρ_U (density ratio $R \equiv (\rho_U - \rho_l)/(\rho_B - \rho_l)$), part of the suspension is buoyant and can convect away. If none of the underlying, negatively buoyant parts of the suspension are entrained by the convection, the sharp interface descends at the theoretical minimum velocity $v_{\min}$. The fraction $X_{\min}$ of the sediment lifted by the convection corresponds in the figure to the value that makes the areas of the two shaded regions equal. Both $v_{\min}(R)$ and $X_{\min}(R)$ are monotonic increasing functions of R , and need to be evaluated numerically⁴ if the volume fraction of particles in the suspension is large enough for hindered settling to be significant.

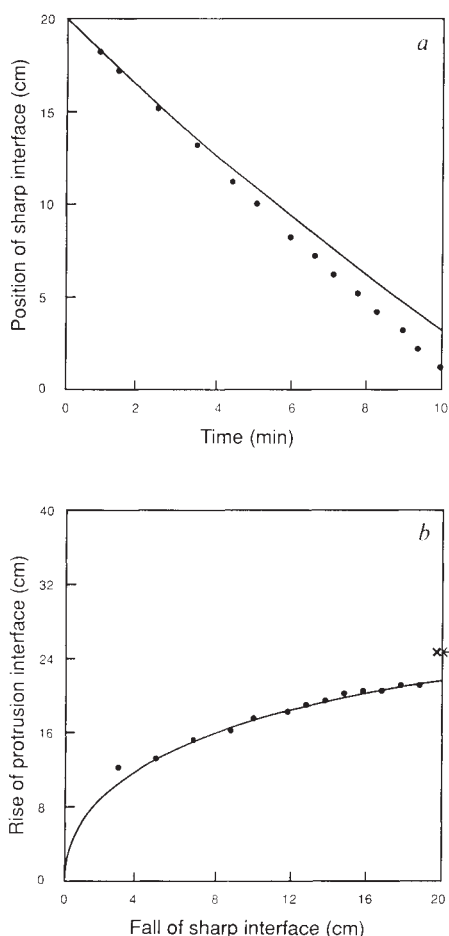


FIG. 4 Experimental values (●) and theoretical curves, predicted from equations (2-4), for the positions of the interfaces during the first cycle (Fig. 1b, c) of simultaneous sedimentation and convection. *a*, Descent of the sharp interface that separates the region of vigorous convection from the underlying region of stagnant sedimentation. The gradual separation of the data from the predicted curve is explained in the text. *b*, Rise, h_g , of the protrusion interface during the fall, h_s , of the sharp interface. One data point (x) indicates the final extent of gradient erosion after several cycles of convection have led to the settling of all the sediment (Fig. 1f). This point is predicted accurately (←) by the simple equation $h_g(\infty) = \sqrt{d_s^2 + 2d_s d_g} - d_s$ that results from setting $X_{\min}$ equal to zero in equation (4), integrating, and replacing h_s by d_s .

Turbidity currents are formed in the ocean by slumping⁶, and by cyclones¹ and large storms⁷ which stir up sand and silt in shallow waters. As the resulting dense suspensions flow into deeper waters they can entrain more sediment, to create self-accelerating turbidity currents⁸. Hence the final suspension that arrives at the sea floor will be a mixture of the sediment properties along the path of the current. Given the content and depth of the final suspension and the nature of the overlying density gradient, the physical model described here accurately predicts the subsequent evolution. In particular, the model determines the rate and extent of erosion of the gradient, as well as the rate of accumulation of the sediment and the variation in particle size distribution within it. In the case of the Sulu Sea¹, the discovery of ~2,000 m of homogeneous deep water underlying a gradient is entirely consistent with the final state (Fig. 1f) observed experimentally.

Similar mixing behaviour is likely to occur in stratified lakes subject periodically to river inputs of light but sediment-rich water⁹⁻¹¹. In both oceans and lakes, the sediment may contain a large proportion of small clay particles. These particles typically coagulate¹²⁻¹⁴ into large flocs that have much greater settling velocities, a process that may further complicate the diverse behaviour¹⁵⁻¹⁶ of dense suspensions of buoyant fluid. □

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A high-pressure form of Al_2SiO_5 as a possible host of aluminium in the lower mantle

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ALTHOUGH iron, magnesium and calcium silicates are the principal components of the lower mantle, the significant amount of aluminium present must also be included in models of mantle geochemistry^{1–4}. The host mineral for aluminium and other trivalent ions is still open to debate. Candidates include a high-pressure form of MgAl_2O_4 (ref. 5), a form of $(\text{Ca}, \text{Mg})\text{Al}_2\text{Si}_2\text{O}_8$ with the hollandite structure⁶ and $\text{Ca}_2\text{AlSiO}_{5.5}$ with a rhombohedral perovskite structure⁷. Here we describe a new candidate formed by transforming $\text{CaAl}_2\text{Si}_2\text{O}_8$ anorthite, $\text{Ca}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ grossular and $\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ pyrope in a diamond anvil cell at 2,500 K and 40–70 GPa. Analytical transmission electron microscopy reveals the formation of a new high-pressure phase of Al_2SiO_5 , for which electron diffraction patterns show strong similarities to the V_3O_5 structure. We estimate that this phase could be present in the lower mantle in a volumetric proportion of about 5%.

Liu⁸ reported that kyanite, one of the three known Al_2SiO_5 polymorphs, dissociates into Al_2O_3 and SiO_2 oxides at pressure above 3 GPa. Birle and Ehlers⁹ synthesized an unidentified high-pressure phase of Al_2GeO_5 at pressures above 3.5 GPa, and suggested that Al_2SiO_5 kyanite probably transforms to denser polymorphs above 15 GPa. A new Al_2SiO_5 high-pressure phase was first observed in the products of transformation of anorthite at 50 GPa, but its structure could not be determined⁶. Our recent results on the products of transformations of $\text{Ca}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ grossular and $\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ pyrope confirm the stability of this new polymorph in the pressure range 40–70 GPa, and allowed the identification of its possible structure type.

To identify the structure of this new alumina high-pressure phase, we have transformed natural Al_2SiO_5 kyanite, as well as natural $\text{CaAl}_2\text{Si}_2\text{O}_8$ anorthite, $\text{Ca}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ grossular and $\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ pyrope, in a laser-heated diamond-anvil cell (the compositions of samples are very close to those of end-members). The starting material, finely crushed in an agate mortar and mixed with a trace amount of platinum to absorb the laser radiation, was loaded in the hole of a nickel gasket. Samples were then brought to high pressures (40–70 ($\pm 10\%$) GPa), as determined with a strain gauge¹⁰, and heated at temperatures $\sim 2,500$ K by scanning the beam of an yttrium–aluminium garnet laser across the sample. After heating, the quenched products were thinned by ion-beam milling and observed with a Jeol 2000 EX electron microscope equipped with a Tracor TN 5400 FX analytical attachment. The transfor-

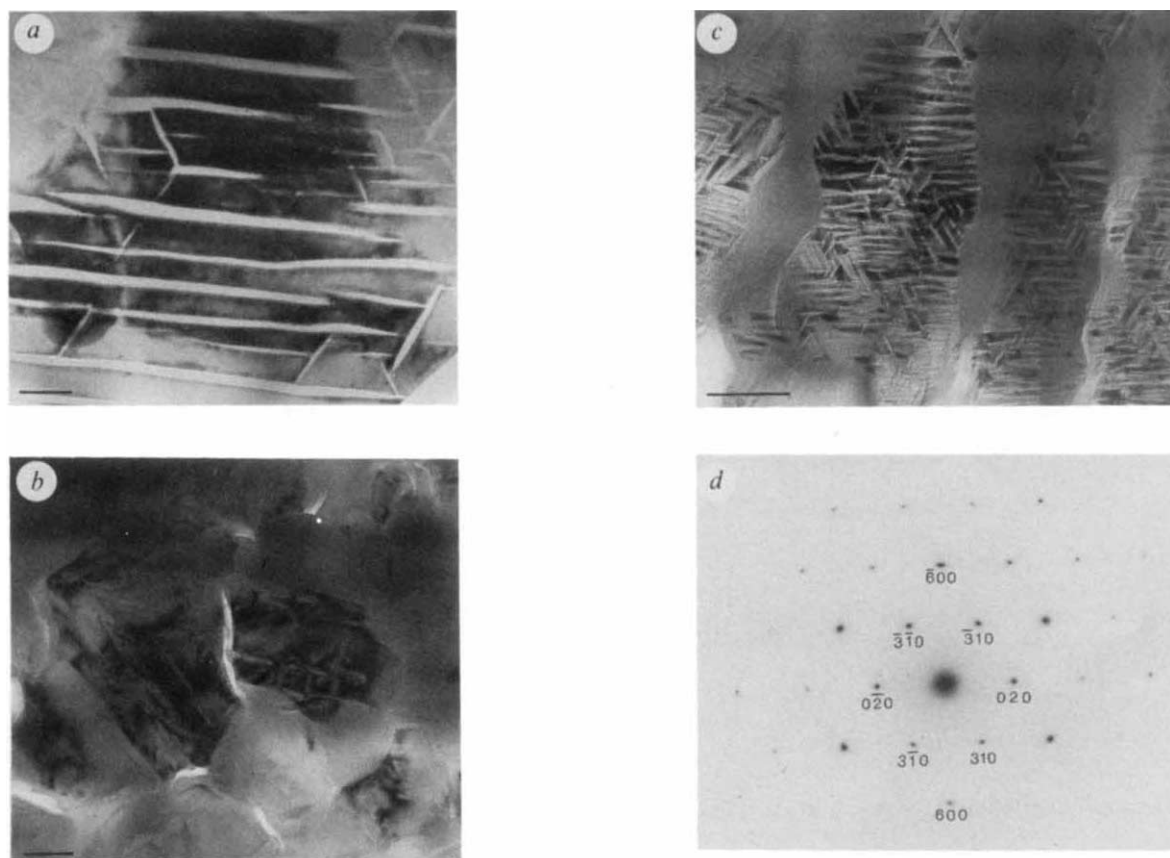


FIG. 1 Transmission electron micrographs. *a*, The Al_2SiO_5 high-pressure phase identified in samples of natural kyanite brought to ~ 50 GPa in a diamond-anvil cell and heated by a laser beam. The grains of the new phase appear as large monocrystals finely striated by amorphous lamellae (in white) of same composition. Scale bar, $0.1\ \mu\text{m}$. *b*, The decomposition of anorthite $\text{CaAl}_2\text{Si}_2\text{O}_8$ at high pressure into a glass of composition CaSiO_3 and the crystalline Al_2SiO_5 high-pressure phase. The glass is assumed to be the result of the amorphization of the CaSiO_3 perovskite. Scale bar,

$0.1\ \mu\text{m}$. *c*, The decomposition of grossular into a glass of composition CaSiO_3 , the crystalline CaO phase and the crystalline Al_2SiO_5 high-pressure phase. The CaO phase (not visible on this micrograph) appears as very fine grains in other regions of the sample. Scale bar, $0.1\ \mu\text{m}$. *d*, Basic electron diffraction pattern taken on the crystalline Al_2SiO_5 high-pressure phase. It corresponds to the $(100)^*$ plane in the reciprocal lattice of the Cr_2TiO_5 structure.

med materials were identified by electron diffraction and/or microanalysis. All experimental procedures, together with the precision of chemical analysis, are described elsewhere in detail^{11,12}.

In all samples, the new Al_2SiO_5 high-pressure phase was observed by transmission electron microscopy (TEM). The chemical composition of the new phase, given by *in situ* X-ray microanalysis, was almost exactly Al_2SiO_5 with small amount of calcium. In some samples, a few small grains with a $\text{Al}_2\text{Si}_2\text{O}_7$ composition were also observed. The new Al_2SiO_5 polymorph appears as a single phase in the products of transformation of kyanite (Fig. 1a), whereas it appears as a product of decomposition of anorthite (Fig. 1b), and garnets (Fig. 1c) at high pressure. The reactions of decomposition of anorthite and garnets are as follows:



In all cases, the new crystalline Al_2SiO_5 polymorph is stable under the electron beam, whereas the CaSiO_3 phase is a glass at room pressure, and the MgSiO_3 phase rapidly amorphizes under the electron beam. The amorphous CaSiO_3 phase identified in the products of transformation of anorthite and grossular is assumed to be the result of the amorphization of CaSiO_3 perovskite, as generally observed¹³. The electron diffraction patterns taken on the MgSiO_3 phase before amorphization are compatible with the structure of orthorhombic perovskite¹⁴. Those obtained on the stable Al_2SiO_5 phase are new (Fig. 1d): they cannot be indexed in the triclinic kyanite structure, nor in other high-pressure structures such as perovskite. In all samples, the new phase has the same texture: Al_2SiO_5 grains appear as relatively large monocrystals (1–2 μm) finely striated with amorphous lamellae of the same composition (Fig. 1a, b, c). The amorphous Al_2SiO_5 lamellae are often orientated at 120° , at a

few degrees from the three main crystallographic directions of the electron diffraction pattern. This particular orientation of lamellae can be related to the possible shear structure of the new phase, as it will be seen below. We note that the other alumina-rich phases (MgAl_2O_4 , $(\text{Ca}, \text{Mg})\text{Al}_2\text{Si}_2\text{O}_8$ and $\text{Ca}_2\text{AlSiO}_{5.5}$) identified previously^{5–7}, were not observed in the products of decomposition of anorthite and garnets. The high-pressure form of Al_2SiO_5 therefore seems to be the most important host mineral for aluminium at high pressure, at least in the pressure range 40–70 GPa and in the $\text{CaO-MgO-Al}_2\text{O}_3\text{-SiO}_2$ system.

X-ray powder diffraction data for the new phase are listed in Table 1. These data were recorded on a kyanite sample quenched from ~ 50 GPa, with a 180-mm Debye-Scherrer camera using $\text{Cu K}\alpha$ radiation. We observed 26 lines. Out of these, 25 can be indexed on the basis of a monoclinic cell with lattice parameters $a = 8.478$, $b = 4.471$ and $c = 6.782$ Å, with $\beta = 104.25^\circ$. The molar volume and the density of the new phase calculated on the basis of four formula units ($Z = 4$), are 150.00 ± 0.08 $\text{cm}^3 \text{mol}^{-1}$ and 4.320 ± 0.003 g cm^{-3} , respectively, at room temperature and pressure. The characteristics of the X-ray reflection extinction ($h0l$ with $l = 2n + 1$) are consistent with the space group $P2_1/c$, with the single exception of the 3.1535-Å line. This line could be due to the presence of untransformed kyanite starting material, preserved at the edge of the sample, since the line corresponds to the most intense 021 reflection of kyanite.

The unit-cell parameters of the new Al_2SiO_5 high-pressure phase are similar to those of V_3O_5 or Cr_2TiO_5 (V_3O_5 -type structure). The indexing and intensities of the main X-ray diffraction lines conform to those of Cr_2TiO_5 . Moreover, the electron diffraction pattern obtained on the new crystalline Al_2SiO_5 phase (Fig. 1d) is similar to that observed on Cr_2TiO_5 (ref. 15). The V_3O_5 -type structure can be derived from the rutile structure by a sequence of crystallographic shears ($\bar{1}21$)¹⁵. This structure can be described as a pseudo-hexagonal close-packing of oxygen

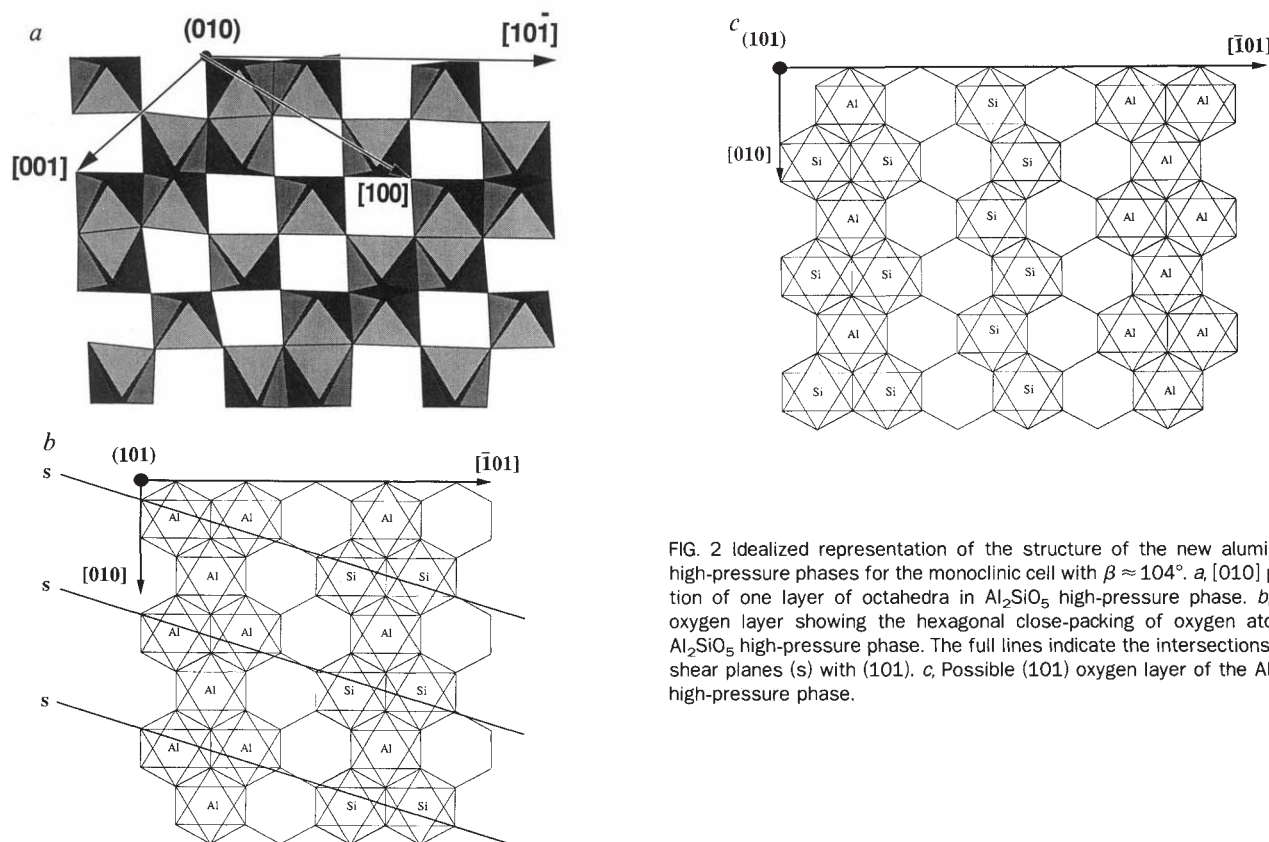


FIG. 2 Idealized representation of the structure of the new alumina-rich high-pressure phases for the monoclinic cell with $\beta \approx 104^\circ$. a, [010] projection of one layer of octahedra in Al_2SiO_5 high-pressure phase. b, (101) oxygen layer showing the hexagonal close-packing of oxygen atoms in Al_2SiO_5 high-pressure phase. The full lines indicate the intersections of the shear planes (s) with (101). c, Possible (101) oxygen layer of the $\text{Al}_2\text{Si}_2\text{O}_7$ high-pressure phase.

atoms with the octahedral interstices partially filled with tri- and tetravalent cations (Fig. 2). The cation-containing octahedra form chains of two types which run parallel to [001] in the (010) plane (Fig. 2a). One is composed of pairs of face-sharing octahedra linked by shared edges; the other is composed of apex-sharing octahedra. The two types of chains are connected by shared apices. When the structure is viewed down [101], oxygen atoms can be seen to form pseudo-hexagonal layers (Fig. 2b). By analogy with Cr_2TiO_5 and V_3O_5 structures, we can consider that the Al_2SiO_5 structure is related to that of rutile by crystallographic shears. In this case, the tetravalent atoms (Si^{4+}) must be preferentially situated at the shear planes in the chains of double octahedra, while the trivalent atoms, Al^{3+} , are situated between the shear planes in the single octahedral chains, or in the empty sites of the shear planes. The tendency of the high-valent (smaller) atoms to reside at the shear planes has been previously observed¹⁶ in several other oxides of the V_3O_5 type (such as V_3O_5 , V_2TiO_5 and Ti_2CrO_5). This general trend does not seem surprising, as a smaller (high-valent) atom should fit more easily into a distorted environment than a larger (lower-valent) one, and the coordination around a cation naturally is less symmetrical at a shear plane than between shear planes in a shear structure. The atom valency distribution could thus be $[\text{Si}^{4+}, \text{Al}^{3+}]_s[\text{Al}^{3+}]_s\text{O}_5$, where s indicates the shear plane. Definitive identification of the structure of the new high-pressure phase will require refinement of single-crystal data, but it is difficult to produce large single crystals at such high pressure.

The possible shear structure of the new phase could explain the origin of the amorphous lamellae seen in all samples (Fig. 1). Indeed, the amorphization of a metastable phase at room pressure and room temperature begins along crystalline defects such as dislocation walls, or twin domains. For instance, the

amorphization of the $(\text{Mg}, \text{Fe})\text{SiO}_3$ perovskite starts along twin boundaries^{11,17}, which are high-energy domains in the structure. The same phenomena could occur in the new phase; the amorphization would start along the shear planes which are domains of higher energy than the surrounding matrix.

Cr_2TiO_5 is a member of an homologous series of phases $\text{Cr}_2\text{Ti}_{n-2}\text{O}_{2n-1}$ ($n=3, 4, 5$) structurally related to the $\alpha\text{-PbO}_2$ structure¹⁵. The latter structure is derived from the rutile structure by a sequence of crystallographic shears $(011)^{15}$. The first member of the series, Cr_2TiO_5 , is isomorphous with V_3O_5 , whereas the structure of the others, $\text{Cr}_2\text{Ti}_2\text{O}_7$ and $\text{Cr}_2\text{Ti}_3\text{O}_9$, may be described as an ordered intergrowth of V_3O_5 and $\alpha\text{-PbO}_2$ structural types. In these compounds, the $\alpha\text{-PbO}_2$ slabs run parallel to [010] and alternate with the V_3O_5 slabs in the [101] direction. By analogy, we expect the Al_2SiO_5 high-pressure phase occasionally observed in our samples to be derived from the Al_2SiO_5 structure by intercalating a SiO_2 slab having the $\alpha\text{-PbO}_2$ structure (Fig. 2c). The monoclinic unit cell of this Al_2SiO_5 high-pressure phase would have the same b and c parameter, but with the a and β parameter given by the periodicity of slabs Al_2SiO_5 and SiO_2 . They are estimated to be $a \approx 10.6 \text{ \AA}$ and $\beta \approx 110^\circ$.

Our observations show that an aluminous phase of composition Al_2SiO_5 with V_3O_5 structure can accommodate aluminium at high pressure, at least in the $\text{CaO-MgO-Al}_2\text{O}_3\text{-SiO}_2$ system. The presence of iron in the starting material could still modify this result. The close-packed structure gives this new phase a very high density, 4.32 g cm^{-3} , which is higher than the densities of Mg-perovskite (4.11 g cm^{-3}) and Ca-perovskite (4.24 g cm^{-3}). It is also $\sim 4.5\%$ denser than the isochemical assemblage of corundum plus stishovite (4.133 g cm^{-3}) at 1 bar. The high density of this Al_2SiO_5 high-pressure phase, and the fact that this phase is found in contact with Mg- or Ca-perovskite, suggest that it could be an important host of aluminium in the deep mantle. Assuming a pyrolytic composition for the lower-mantle, one can predict that the proportion of the Al_2SiO_5 high-pressure phase, in volumetric ratio, is $\sim 5\%$. Its presence would probably not cause significant changes in lower-mantle seismic velocities.

Recent X-ray investigations of high-pressure samples (50 GPa) of peridotite¹⁸ suggest that only $(\text{Mg}, \text{Fe})\text{SiO}_3$ perovskite, CaSiO_3 perovskite and $(\text{Mg}, \text{Fe})\text{O}$ magnesio-wüstite are stable at this pressure. No aluminium-bearing phase was mentioned, but this may be because the aluminous phases are present in small amounts which are not resolved by powder X-ray investigation. We thus suggest that electron microscopy is better suited to the identification of minor phases in samples synthesized in a diamond-anvil cell. □

TABLE 1 X-ray powder diffraction data for the Al_2SiO_5 high-pressure phase

<i>h</i>	<i>k</i>	<i>l</i>	<i>d</i> _{obs}	<i>d</i> _{calc}	<i>I</i> / <i>I</i> ₀
0	1	1	3.7231	3.6965	<5
0	0	2	3.2903	3.2819	8
0	2	1	3.1535*	kyanite	
2	0	-2	2.9402	2.9423	14
1	1	-2	2.6827	2.6790	6
2	1	1	2.5779	2.5758	<5
3	1	0	2.3382	2.3358	32
0	2	0	2.2379	2.2367	100
3	1	-2	2.1366	2.1260	7
4	0	0	2.0579	2.0541	11
1	2	1	2.0144	2.0152	21
2	2	-1	1.9450	1.9460	64
4	1	-1	1.9103	1.9118	<5
3	2	-1	1.7430	1.7435	13
3	2	0	1.7217	1.7248	6
2	0	-4	1.6695	1.6726	<5
4	0	2	1.5752	1.5752	18
0	3	0	1.4903	1.4911	7
1	3	0	1.4650	1.4672	7
6	0	0	1.3680	1.3694	53
2	3	-2	1.3308	1.3301	11
3	0	4	1.2780	1.2790	<5
0	3	3	1.2316	1.2322	6
4	3	0	1.2056	1.2067	<5
3	3	2	1.1715	1.1698	21
7	1	-3	1.1240	1.1232	8

Calculated from $a=8.478(7)$, $b=4.471(3)$, $c=6.782(8) \text{ \AA}$ and $\beta=104.25(9)^\circ$ for the monoclinic V_3O_5 structure. Molar volume $V=248.98 \text{ \AA}^3$ ($=150.006 \text{ cm}^3 \text{ mol}^{-1}$), $\rho=4.320 \text{ g cm}^{-3}$. Lines can be indexed for space group $P2_1/c$.

* The reflection at $3.1535 \text{ \AA}$ probably corresponds to the most intense reflection (021) of untransformed kyanite starting material.

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Iso-orientation domains in cat visual cortex are arranged in pinwheel-like patterns

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THE mammalian cortex is organized in a columnar fashion: neurons lying below each other from the pia to the white matter usually share many functional properties. Across the cortical surface, cells with similar response properties are also clustered together, forming elongated bands or patches. Some response properties, such as orientation preference in the visual cortex, change gradually across the cortical surface forming 'orientation maps'. To determine the precise layout of iso-orientation domains, knowledge of responses not only to one but to many stimulus orientations is essential. Therefore, the exact depiction of orientation maps has been hampered by technical difficulties and remained controversial for almost thirty years. Here we use *in vivo* optical imaging based on intrinsic signals to gather information on the responses of a piece of cortex to gratings in many different orientations. This complete set of responses then provides detailed information on the structure of the orientation map in a large patch of cortex from area 18 of the cat. We find that cortical regions that respond best to one orientation form highly ordered patches rather than elongated bands. These iso-orientation patches are organized around 'orientation centres', producing pinwheel-like patterns in which the orientation preference of cells is changing continuously across the cortex. We have also analysed our data for fast changes in orientation preference and find that these 'fractures' are limited to the orientation centres. The pinwheels and orientation centres are such a prominent organizational

feature that it should be important to understand their development as well as their function in the processing of visual information.

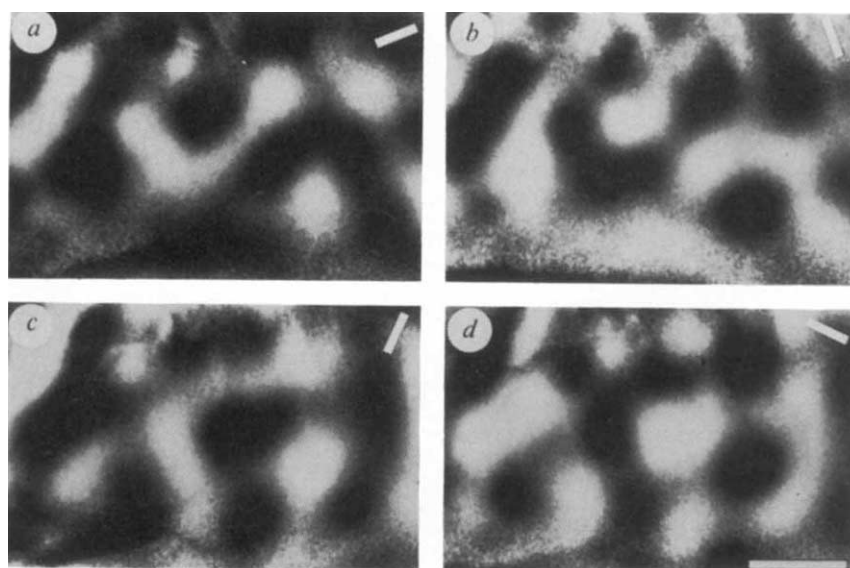
The aim of this study was to determine organizational principles underlying orientation preference maps. There has been controversy about how orientation is exactly organized in visual cortex. The original conjecture of Hubel and Wiesel^{1,2} was that orientation maps are composed of "swirling stripes with many bifurcations and endings"². Subsequently other investigators^{3,4} proposed that many of the data obtained by Hubel and Wiesel could also be explained by centres around which the different orientation domains are organized in a pinwheel-like fashion. Until recently it has not been possible to distinguish reliably between these two possibilities as experiments either lacked a sufficient spatial resolution or were carried out by only looking at the cortical responses to gratings of one or two orientations, thus yielding an incomplete picture subject to multiple interpretations.

We used *in vivo* optical imaging based on intrinsic signals⁵ to map the orientation preference of cat area 18 as this technique allows the recording of many high-resolution functional maps from one cortical site. Figure 1 shows four such orientation maps obtained with moving gratings of different orientations as stimuli. The dark patches depict regions most strongly activated by the respective stimuli. The four panels show the characteristic orientation maps which are reminiscent of beaded 2-deoxyglucose maps^{2,6}. These patterns have usually been interpreted as bands of iso-orientation domains.

In contrast to the 2-deoxyglucose method, optical imaging of intrinsic signals now enables us to determine the response properties of the greyish 'interbead regions'. Careful comparison of the different parts of Fig. 1 shows that every interbead region in one picture is complemented by a dark bead in another orientation map, usually $\sim 45^\circ$ different. This indicates that the banded structure in the 2-deoxyglucose pictures and in the four optical maps of Fig. 1 may be deceptive: the iso-orientation domains are not elongated bands with a beaded appearance, but are rather patchy, with the greyish interbead regions not belonging to the same iso-orientation domain as the dark beads. They actually correspond to beads of the neighbouring orientation.

FIG. 1 Optical imaging of iso-orientation columns in area 18 of cat visual cortex. *a-d*, Four activity maps, each produced by a moving grating of a particular orientation (displayed in the upper right corner of each panel). Areas that appear dark in the picture are the regions in which the response to the respective stimulus is maximal. The characteristic patterns in all four maps is one of beaded bands⁶, dark regions of very strong activation (beads) connected by lighter 'interbead regions'. The size of the mapping signal^{2,6} (that is, the relative reflectance change $\Delta R/R$ in a black point minus the value of $\Delta R/R$ in a white point) in the four panels is 0.0015. Scale bar, 1 mm.

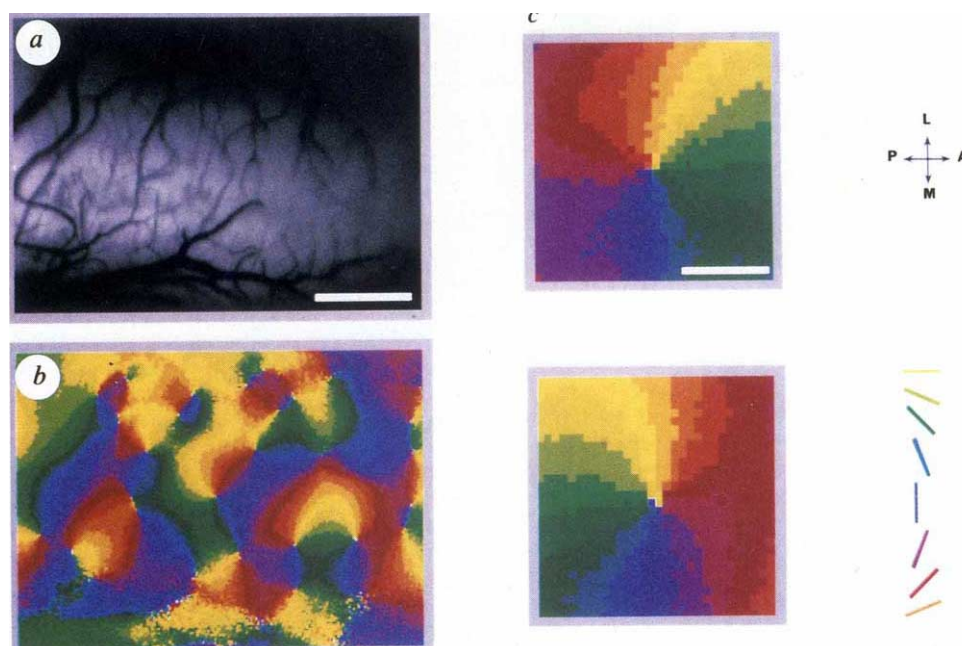
METHODS. Cats were anaesthetized (sodium pentothal) and paralysed (succinyl choline-HCl) using standard procedures. The skull of the cat was opened above area 18 at Horsley-Clarke A5. A stainless steel chamber was cemented onto the skull, the dura removed, and the chamber filled with silicone oil and sealed with a round glass coverslip. For more details, see refs 16–18. Large field (40° by 40°) visual stimuli were presented binocularly on a video screen (Mitsubishi) in 60 Hz non-interlaced mode. They consisted of gratings of eight different orientations with a spatial frequency of 0.15 cycles per degree moving at a speed of 15 deg s^{-1} . A slow-scan charge-coupled device camera (Photometrics) acquired images from the activated cortex for every stimulus condition separately (with the eight different orientations presented in a random sequence). To achieve a better signal-to-noise ratio, every response to a different stimulus condition was averaged 10 to 100 times. Our functional maps were obtained by dividing the picture acquired



for each stimulus by the sum of all the responses (thereby correcting for the effects of uneven illumination). The pictures in Figs 1, 2 and 3 all result from this algorithm, which requires a good signal-to-noise ratio of the optical maps. In other studies^{5,10,16} where contrast enhancement was desired, the picture obtained with one stimulus was divided by that obtained with the orthogonal stimulus.

FIG. 2 Pinwheel-like structures are a prominent feature of the functional organization of iso-orientation domains. *a*, An image of the region of cortex explored for its organization of orientation preferences. The microvasculature is clearly visible in this picture. Scale bar, 1 mm. *b*, Colour-coded orientation preference map in cat area 18. The preferred orientation for every location is coded according to the scheme shown on the right; areas responding best to a horizontal bar are coded in yellow; areas responding best to a vertical bar are coded in blue, and so on. It is apparent that the overall organization is patchy rather than banded. Pinwheel-like structures around orientation centres are a prominent feature in this map. *c*, Close-up of two orientation centres. Two fundamental features of the orientation centres are illustrated: (1) in all 'pinwheels', each orientation appears once around the centre; (2) the pinwheels exist in two forms: a clockwise form (upper), in which the sequence of the colours yellow-green-blue-red is clockwise, and a counterclockwise form (lower), in which the inverse is the case. Scale bar, 300 μ m. A, anterior; P, posterior; M, medial; L, lateral.

METHODS. For the vectorial analysis we took the activity maps recorded with eight different orientations and (after multiplying the angles by two)^{7,19} added them vectorially on a pixel by pixel basis^{10,17}. The resulting vector

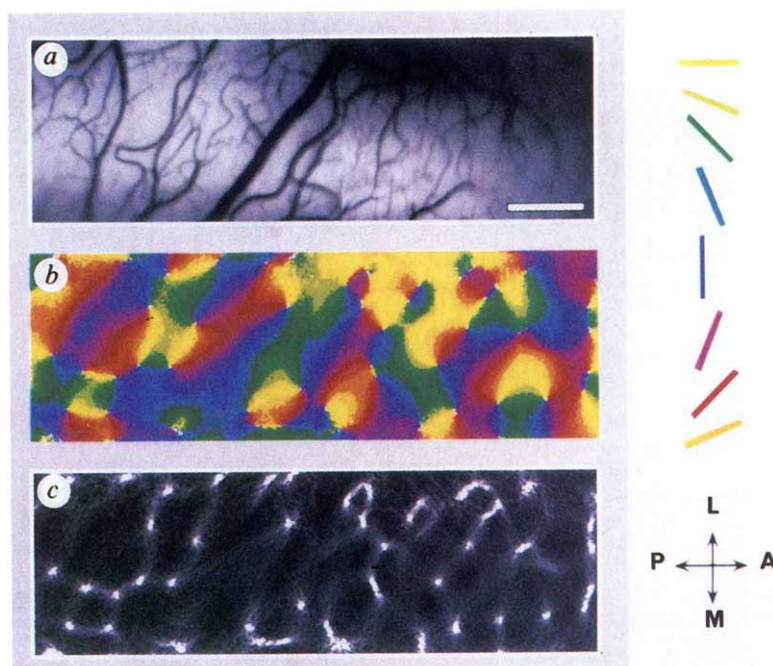


angle (again divided by two) was then colour-coded. The pictures in *b* and *c* (and in Fig. 3*b*) were not subjected to any smoothing algorithm. But, we used only 16 colours so that, for instance, the preferred angles between 0° and 12.5° are all coded in yellow.

Optical data as presented in the individual panels Fig. 1 give an incomplete picture of the organization of orientation preference. The correct assessment of the organization of orientation preference is only possible by comparing the activity maps from many stimulus orientations. In order to get a complete view of the overall architecture of orientation preference in area 18, we therefore combined maps resulting from eight different stimulus orientations. For every point in the imaged cortex we then calculated to which orientation the underlying cells responded best. The results from this vectorial analysis are shown in Fig. 2.

The angle of best response is coded with colours according to the scheme shown at the right of the picture. This colour map of orientation preference now gives a complete picture of how this stimulus attribute is organized in cat area 18. The data indicate that regions preferring similar orientations are not bands, but rather appear to form patches which can be up to 0.5 mm² in size (if coded with 22.5° resolution). These iso-orientation patches are invariably organized so that orientation is mapped in a continuous, pinwheel-like fashion around vortices, or 'orientation centres'. This result confirms and extends

FIG. 3 'Orientation centres' and the rate of orientation change across the cortical surface. *a*, An image of a long stretch of area 18 which was examined in this experiment. Scale bar, 1 mm. *b*, Orientation preference pattern of this piece of cortex. The colour coding is again shown at the right. This map is derived from the responses to gratings of eight different orientations. To test whether orientation changes within the pinwheels are smooth we also produced a map using only four orientations and interpolations (not shown). The interpolated map was very similar to the map obtained with the full eight orientations. This result indicates that orientation changes away from the centres are rather gradual and smooth. *c*, 'Fracture map': we screened our data for the locations with the strongest orientation change by applying a gradient operator to the orientation map. We calculated the two-dimensional derivative (taking the circular nature of the orientation data into account) for every point of the picture and displayed its absolute value on a grey scale. White elements in the maps then showed the regions of strong orientation changes. Evidently the regions where orientation changes are strongest are the orientation centres manifesting themselves as small white patches. Note that there are no rapid changes along lines, except at the periphery of the map, where the cortex is already folding down, and where the data are much noisier. The data presented were accumulated in two independent experiments run ~4 h apart on adjacent pieces of cortex. In *a*, *b* and *c* a slight break is seen where the data of the two different experiments were pasted together.



earlier findings⁷ in which multiple electrical penetrations (with a spacing of 200–300 μm) and interpolation algorithms were used to derive orientation maps. It is in principle difficult, however, to draw detailed conclusions about singularities from interpolated data.

We found 1.2 of the orientation centres per mm^2 on average. In Fig. 2c, two typical orientation centres are shown at a higher magnification. The different domains of continuously changing orientation encircle the centres, with every orientation appearing once around the centre singularity. We found a total of 120 orientation centres, and in each the sequence of the different orientation domains appeared once around the centre singularity (thus mapping the 180° orientation change onto a 360° circle). Figure 2c also shows that the orientation centres appear in two different forms: one in which the sequence of the colours (and thus preferred orientations) is clockwise (upper part) and one in which it is counterclockwise (lower part of Fig. 2c). Out of the 12 hemispheres analysed in this manner, only two (which were recorded rather posteriorly) showed a banded structure. The remaining 10 hemispheres displayed the remarkable radial organization. This indicates that 'pinwheels' are prominent organizational features in area 18 of cat visual cortex.

Sudden jumps of orientation preference have been reported in tangential electrophysiological recordings^{8,9}. To establish whether these jumps correspond to the traversing of one of the orientation centres, or if there are additional cortical loci where orientation changes very abruptly, we analysed our data with respect to the slope of orientation change. Figure 3 shows the blood vessel pattern (Fig. 3a) and a colour-coded orientation map of a long strip of area 18 (Fig. 3b); in Fig. 3c the rate of orientation change for this piece of cortex is shown: the loci of the most rapid changes are coded in white, whereas a black region signifies slow orientation change. By comparing Fig. 3b and c, the regions of strongest orientation change—represented by the small white patches—can be seen to coincide with the orientation centres. Orientation changes are much slower in the other regions. Although we noticed that several lines connecting different orientation centres show slightly stronger orientation change (they appear as greyish lines), there are no lines along which orientation changes abruptly, or 'fractures', as reported for monkey V1 (ref. 10).

Several of our optical maps were confirmed with electrode recordings at multiple sites. We were interested in determining whether single units at the orientation centres are more broadly tuned than elsewhere. We made single and multi-unit recordings at three to six sites in the vicinity of the orientation centres mapped optically (14 orientation centres in two cats). Cells at (or close to) the orientation centres had normal orientation tuning. However, the optimal orientation changed quickly over short distances.

The organization experimentally observed in area 18 is remarkably similar to some theoretical models for the organization of area 17 (refs 3, 4), in which it was proposed that orientation might be organized in a circular rather than in a banded fashion, with every orientation appearing twice, or in later models^{11–15} only once, around the centre singularity. Figure 2b and c shows that the later models agree better with experimental reality.

We conclude that the orientation centres from which the pinwheel-like organization of orientation preference originates are an important feature for organizing the representation of orientation in area 18. It will be worthwhile to search for anatomical and histological landmarks coinciding with the orientation centres and to characterize the response properties of single cells located in these cortical sites in detail. □

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WT1 mutations contribute to abnormal genital system development and hereditary Wilms' tumour

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WILMS' tumour (WT), aniridia, genitourinary abnormalities and mental retardation form a symptom group (WAGR syndrome) associated with hemizygous deletions of DNA in chromosome band 11p13 (refs 1, 2). However, it has not been clear whether hemizygosity at a single locus contributes to more than one phenotype. The tumour suppressor gene for Wilms' tumour, WT1, has been characterized^{3,4}: it is expressed at high levels in the glomeruli of the kidney⁵, as well as the gonadal ridge of the developing gonad⁶, the Sertoli cells of the testis⁶ and the epithelial and granulosa cells of the ovary⁶, suggesting a developmental role in the genital system in addition to the kidney. We now report constitutional mutations within the WT1 genes of two individuals with a combination of WT and genital abnormalities as evidence of a role for a recessive oncogene in mammalian development.

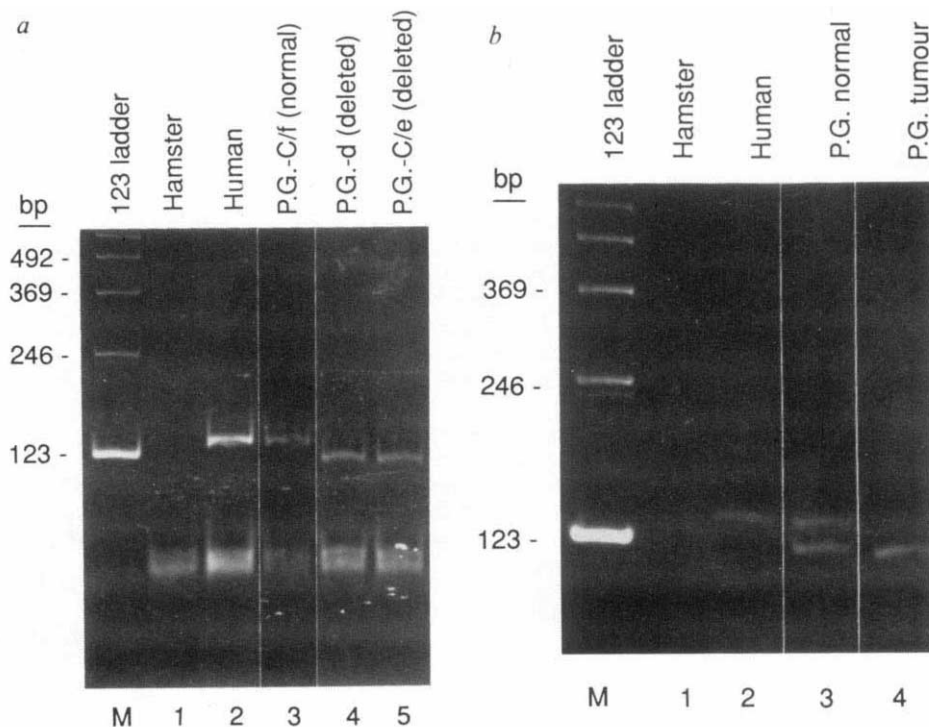
One patient (P.G.) had bilateral Wilms' tumour, hypospadias, and an undescended left testicle. The second patient (T.S.) was born with hypospadias and bilateral cryptorchidism and had recently developed a Wilms' tumour. We have established hamster-human somatic cell hybrids which segregate P.G.'s chromosome 11 (ref. 7). Polymerase chain reaction (PCR) products were analysed on nondenaturing 10% polyacrylamide gels for all 10 exons of the WT1 gene from the segregated chromosomes 11 of patient P.G. A small deletion within exon 4 was observed in the PCR product from the chromosome 11 which was retained in P.G.'s first Wilms' tumour (110 base pairs (bp)) compared with normal controls (~130 bp) (Fig. 1a: compare lanes 4 and 5 with lane 3). Specimens of the second tumour are available

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FIG. 1 Patient P.G. had no family history of urogenital anomalies or Wilms' tumour and no evidence of ocular abnormalities or mental retardation. Karyotype findings were normal (46XY). *a*, PCR analysis of human *WT1* exon 4 from hamster, human, and hamster/human (P.G.) hybrid genomic DNA. Ethidium bromide-stained 10% nondenaturing polyacrylamide gel shows PCR products obtained with oligonucleotides targeted to human *WT1* exon 4. These oligonucleotides are expected to yield a PCR product of 127 bp. The size markers (bp) in lane M are from a 123 sizing ladder (BRL). PCR was performed with a DNA thermocycler (Perkin-Elmer-Cetus) using 0.1 µg genomic DNA in a total volume of 100 µl. Thirty cycles of amplification were carried out, each cycle consisting of 1 min at 94 °C, 2 min at 57 °C, and 2 min at 72 °C. Following completion of the PCR, a 10 µl aliquot was analysed by gel electrophoresis. Genomic DNA samples were from the following sources: (1) hamster cell line CHTG 49; (2) an unrelated human; hamster/human (P.G.) hybrids (3) P.G.-C/f (contains P.G.'s normal 11p haplotype); (4) P.G.-d (contains P.G.'s deleted WT 11p haplotype), and (5) P.G.-C/e (contains P.G.'s WT deleted 11p haplotype). P.G.-d and P.G.-C/e are independently derived somatic cell hybrids. The oligonucleotide primers were WT(4)S: 5'-CCTGAATTCAGTGTGTATATTGTGG-3', and WT(4)AS: 5'-GGCTCCTAAGTTCATCTGATTCC-3'. PCR analysis of >30 normal human DNA samples revealed only the ~130-bp product (J.P., data not shown). *b*, PCR analysis from exon 4 of P.G.'s second WT embedded in paraffin. Sections (10 µm) taken from P.G.'s diaphragm (normal tissue) and a specimen of his second WT were scraped (~5 mm²) from a glass microscope slide into an Eppendorf



centrifuge tube, then deparaffinized by washing twice in xylene, once in 95% ethanol and once in 70% ethanol, and air-drying. Half of the sample was used for PCR¹⁵. Size markers in lane M as in *a*. Lane 1, PCR products from hamster genomic DNA; lane 2, PCR products from human genomic DNA; lane 3, PCR products from adjacent normal tissue (diaphragm); lane 4, PCR product from P.G.'s second Wilms' tumour.

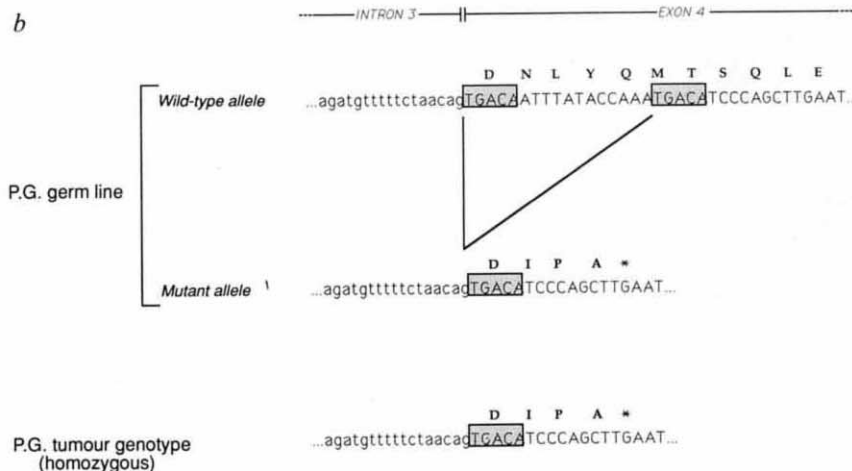
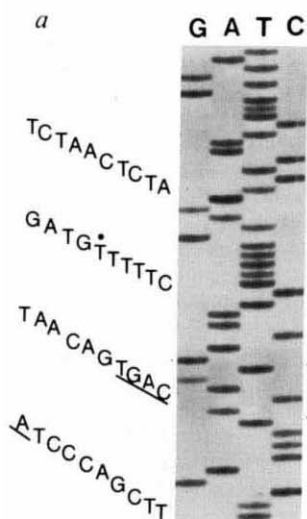
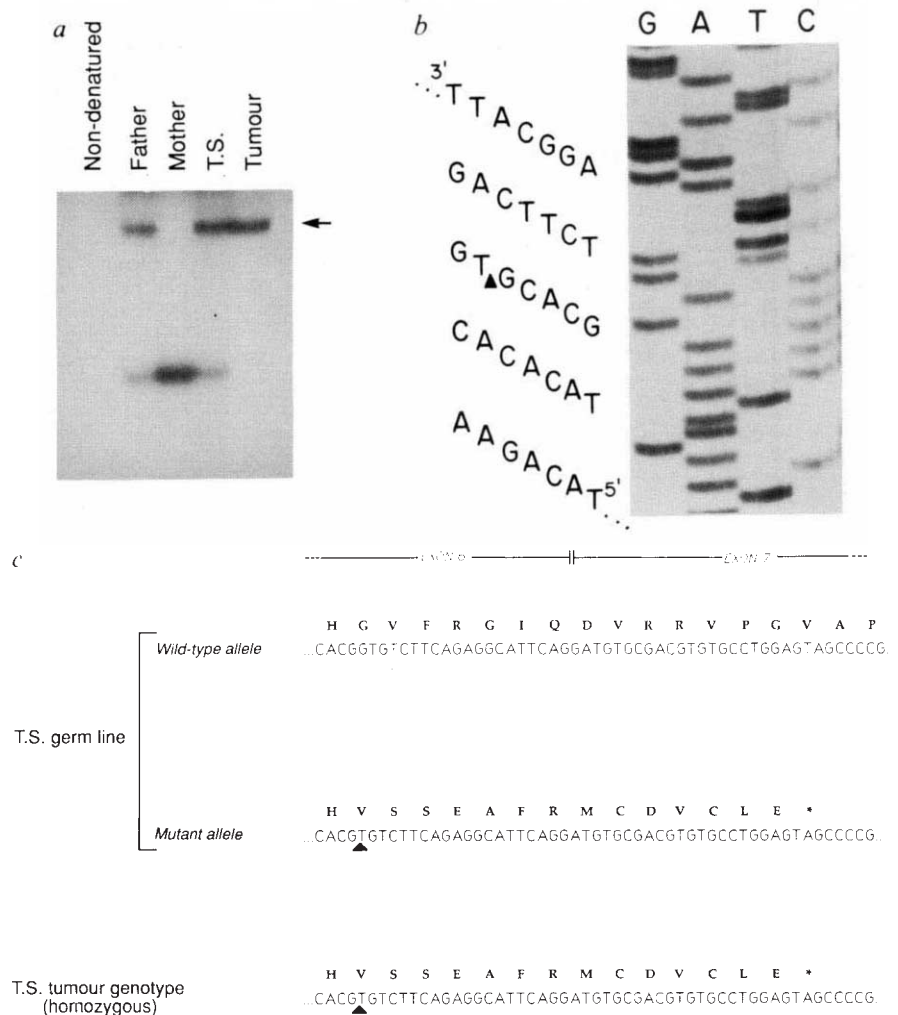


FIG. 2 *a*, Nucleotide sequence analysis of PCR products obtained from P.G.'s 11p WT haplotype. PCR products were obtained by amplification of *WT1* exon 4 from hamster/human hybrid P.G.-C/e (which contains P.G.'s WT 11p haplotype) genomic DNA, gel-purified, treated with T4 DNA polymerase, and subcloned into the *EcoRV* restriction site of pBluescript II KS+ (Stratagene). DNA sequences from 6 subclones (3 from P.G.-C/e; 3 from P.G.-d) were determined by the dideoxy chain termination method¹⁶. All subclones analysed had identical sequences. Direct sequencing primers of pBluescript II

KS+ were obtained from US Biochem. The dot above the T in the sequence reflects a sequence polymorphism within intron 3 of *WT1* (D.A.H. *et al.*, unpublished). *b*, Nucleotide and amino-acid sequence (one-letter code) of human *WT1* exon 4 resulting from P.G.'s 17-bp deletion. The predicted messenger RNA structure contains a frameshift which is expected to cause early termination of translation. The repeated sequence TGACA is boxed. The asterisks denote the TGA stop codon.

FIG. 3 a, Single-strand conformational polymorphism (SSCP) analysis of *WT1* exon 6 in patient T.S. and his parents. PCRs were performed with primers WT(6)S, 5'-CCCCAAGCTT-CACGTGACCCCTTTTCCCTC-3' and WT(6)AS, 5'-CCCCGAATTCCAAGAGTCCATCAGTAAGG-3', amplifying a 238-bp fragment. Conditions for the PCR reaction were as described for Fig. 1, except that the annealing temperature was 60 °C. WT(6)AS was radiolabelled with T4 polynucleotide kinase (New England Biolabs) and [γ - 32 P]ATP (New England Nuclear) before the PCR reaction. Samples of 2 μ l were resuspended in 8 μ l 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol and boiled for 5 min. Samples (2 μ l) were loaded onto an 8% polyacrylamide (50:1, acrylamide:bis-acrylamide) gels and electrophoresed at 30 W in the cold room. The fragment with altered mobility is denoted by an arrow. Thirty normal human DNA samples were tested as controls and no variations were detected (J.P., data not shown). **b**, Nucleotide sequence analysis of PCR products obtained from T.S.'s tumour. The PCR products obtained by amplification of *WT1* exon 6 from T.S.'s Wilms' tumour were subcloned and sequenced as described in the legend to Fig. 2. Four independently derived subclones from T.S.'s tumour were analysed and yielded the sequence presented. Sequencing directly from the PCR product confirmed these results. Elution of the radiolabelled DNA fragment from T.S. that showed altered mobility in **a**, followed by amplification by PCR and subcloning and sequencing yielded the same sequence. The arrowhead represents the site of the guanosine deletion. **c**, Nucleotide and amino-acid sequence (one-letter code) of human *WT1* exon 6 and 7 resulting from T.S.'s deletion. The predicted mRNA structure contains a frameshift that is expected to cause early termination of translation. The asterisks indicate the TGA stop codon.



only as paraffin-embedded blocks, but PCR analysis of exon 4 from the surrounding normal tissue yielded both the ~130-bp and the 110-bp PCR product (Fig. 1b, lane 3), consistent with P.G.'s predicted genotype. Analysis of tumour tissue revealed the presence of only the 110-bp fragment (Fig. 1b, lane 4). We conclude that for both of P.G.'s Wilms' tumours, reduction to homozygosity for the *WT1* allele containing a small deletion in exon 4 contributed to tumour formation. Sequence analysis of the abnormal allele revealed a 17-bp deletion, predicted to cause premature polypeptide chain termination in exon (Fig. 2). The deletion occurred between two copies of the pentanucleotide sequence 5'TGACA3' (Fig. 2b). P.G.'s germline mutation thus appears to be the consequence of either polymerase skipping during DNA replication or an unequal crossover event.

The second patient, T.S., with hypospadias and bilateral cryptorchidism, developed a Wilms' tumour at the age of three years. His father had been successfully treated for Wilms' tumour in 1959. Southern blot analysis failed to detect any deletions within *WT1* for patient T.S. or his parents (J.P. unpublished data). Analysis of PCR-amplified exons on nondenaturing polyacrylamide gels run to detect single-strand conformational polymorphisms⁸ (Fig. 3a) revealed a significant difference in the mobility pattern of exon 6. Three patterns were detected: one for the unaffected mother; a second for T.S. and his father; and a third for T.S.'s tumour (Fig. 3a). No detectable shift was observed in single-strand conformational polymorphism analysis for any other exon from T.S.'s tumour. Sequence analysis (Fig. 3b) revealed that T.S. has a single nucleotide deletion within exon 6, which is predicted to cause early termination of translation (Fig. 3c).

Hereditary Wilms' tumours such as those observed in P.G. and T.S. account for fewer than 5% of all WT cases, but were essential in formulating the two-mutation model of tumorigenesis⁹. Our findings in patients P.G. and T.S. support this model. The exclusion of 11p linkage in four Wilms' tumour pedigrees¹⁰⁻¹² suggests that mutations at a second locus can predispose to Wilms' tumour. But the demonstration of a transmitted *WT1* mutation in T.S. and his father, to our knowledge, documents the first example of the involvement of the 11p13 locus in familial Wilms' tumour.

The developmental circuitry for human sexual development in which *WT1* participates is of great interest. The possibility that *WT1* has a transcriptional relationship with müllerian inhibiting substance⁶, or with any of the genes controlling testosterone biosynthesis and action, or with the Y chromosome gene which initiates male sexual development^{13,14}, should be investigated. Our results indicate that genital anomalies in WAGR individuals are a dominant phenotype of null mutations to *WT1*, and underscores the sensitivity of the developing urogenital system to *WT1* gene dosage. Further reduction of *WT1* expression could occur in hemizygous individuals if the normal allele is subject to germ-line imprinting or positive-feedback regulation. Our results raise the interesting possibility, currently under investigation, that germ-line hemizygous inactivation of *WT1* may result in apparently non-WT associated genital anomalies. □

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***In vivo* cell-specific expression of the cystic fibrosis transmembrane conductance regulator**

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CYSTIC FIBROSIS (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR)^{1–3}. The principal manifestations of CF include increased concentration of Cl[−] in exocrine gland secretions^{4,5}, pancreatic insufficiency, chronic lung disease, intestinal blockage and malabsorption of fat^{5,6}, and male and female infertility⁷. Insight into the function of CFTR can be gained by correlating its cell-specific expression with the physiology of those cells and with CF pathology. Determination of CFTR messenger RNA in rat tissues by *in situ* hybridization shows that it is specifically expressed in the ductal cells of the pancreas and the salivary glands. In the intestine, decreasing gradients of expression of the CFTR gene are observed on both the crypt–villus and the proximal–distal axes. This expression is consistent with CFTR being responsible for bidirectional Cl[−] transport, secretion in the intestinal crypts⁸ and reabsorption in the salivary gland ducts⁴, and suggests that in these tissues CFTR functions as a regulated Cl[−] channel. In the lung, a broad band of hybridization includes the mucosa and submucosa of the bronchi and bronchioles. In the testis, CFTR expression is regulated during the cycle of the seminiferous epithelium. Post-meiotic expression is maximal in the round spermatids of stages VII and VIII, suggesting that CFTR plays a critical role in spermatogenesis and that deficiency of this function contributes to CF male infertility.

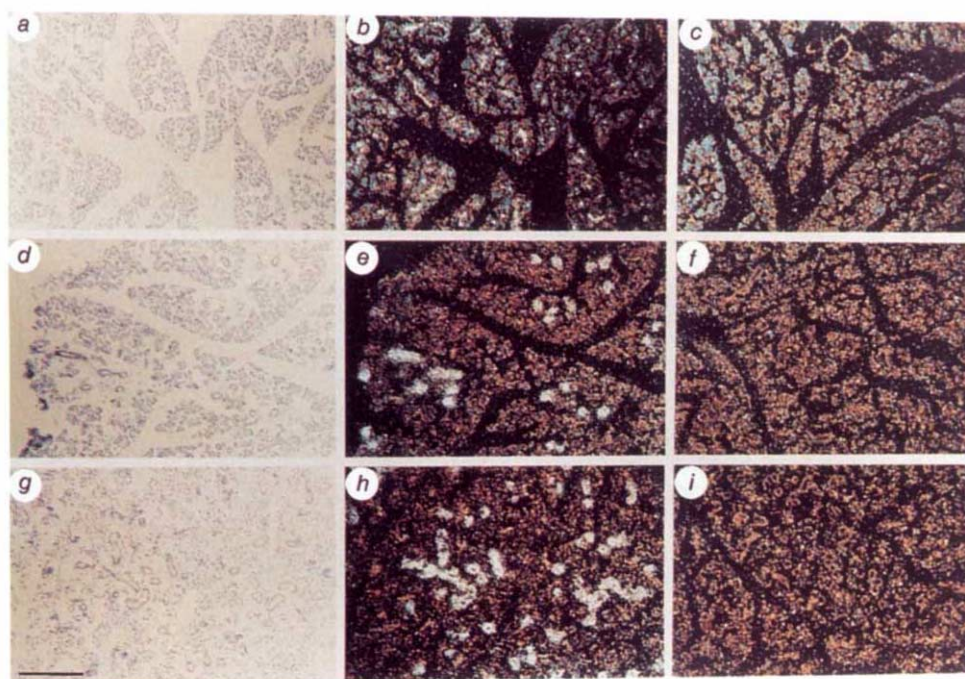
In situ hybridization analyses were performed with ³⁵S-labelled sense and antisense RNA probes synthesized from portions of a rat complementary DNA clone (see legend to Fig. 1 for details). In the pancreas and salivary glands, ductal expression of CFTR (Fig. 1) provides molecular support for the generally held view that these structures are involved in the disease pathology. Lack of ductal modification of the secreted fluid would account for the increase in Cl[−] concentration observed in the salivary gland secretions of CF patients^{4,9}. Expression of CFTR in the rat pancreas is reduced compared with human pancreas², which reflects species differences in the pattern of CFTR expression. Other differences may also exist.

FIG. 1 Photomicrographs of haematoxylin-stained frozen sections of rat pancreas (a, b and c), parotid (d, e, f) and submaxillary glands (g, h and i). a, d and g Bright-field views of sections hybridized to anti-sense ³⁵S-labelled CFTR RNA probes; b, e, h, dark-field views of the same sections. CFTR expression is limited to the centro-acinar cells of the intercalated ducts of the pancreas and CFTR mRNA is undetectable in the surrounding serous acini of the pancreas or in the islets of Langerhans (not shown). In the parotid and submaxillary glands, CFTR mRNA is again restricted to the intercalated and intralobular ducts with no expression in the serous and mucous acini of these glands. c, f and i, Dark-field views of sections hybridized with sense-strand ³⁵S-labelled CFTR RNA. These control hybridizations are negative, with an even low background distribution of silver grains over the entire slide. Scale bar, 200 µm. A partial cDNA of 1.5 kilobases (kb), encoding a region homologous to exons 10 to 16 in the human CFTR cDNA, was generated from rat parotid gland RNA. Two overlapping CFTR cDNA subclones were used to synthesize the ³⁵S-labelled riboprobes used for *in situ* hybridization. These clones encode a portion of the rat homologue of human CFTR, as determined by DNA sequence comparisons, northern blot analysis and their use to map the chromosomal location of rat CFTR (manuscript in preparation). Assuming rat CFTR is interrupted by intronic DNA in the same positions as human CFTR, probe 1 (antisense) and probe 2 (sense) encompass the last 141 base pairs (bp) of exon 10, all of exons 11 and 12, and the first 210 bp of exon 13. Probe 3 (antisense) and probe 4 (sense) encompass the last 45 bp of exon 11, all of exon 12 and the first 210 bp of exon 13. Control hybridizations with probes 2 and 4 produced identical results. We observed slight differences in the overall intensity of the signal produced by probes 1 and 3 and have attributed this to variation in the efficiency of riboprobe synthesis. For each tissue we examined a minimum of 6 sections hybridized with an antisense probe and 6 sections hybridized with a sense probe. *In situ* hybridization was essentially as described²⁸. Cryostat sections (6 µm) of paraformaldehyde-perfused tissue were digested with proteinase K and hybridized in 50% formamide at 55 °C to 60 °C overnight. Excess probe was removed by digestion with RNase A and washing with 0.1 × SSC at 60 °C. Slides were hand-dipped in Kodak NTB2 emulsion, exposed for two weeks at 4 °C, developed and stained with haematoxylin.

In the lung (Fig. 2), CFTR is expressed at low levels in both the surface epithelium and the underlying lamina propria of the bronchi and bronchioles; the levels of expression are similar to those in human respiratory epithelia¹⁰. Expression of CFTR in the submucosa has profound implications for the suitability of somatic gene therapy in CF, as proposed by Rosenfeld *et al.*¹¹. These authors have demonstrated the *in vivo* transfer of a recombinant α -1-antitrypsin gene to the lung epithelium. As CFTR mRNA is also observed in the underlying lamina propria, expression of recombinant CFTR only in the lung epithelium may not be a sufficient form of therapy.

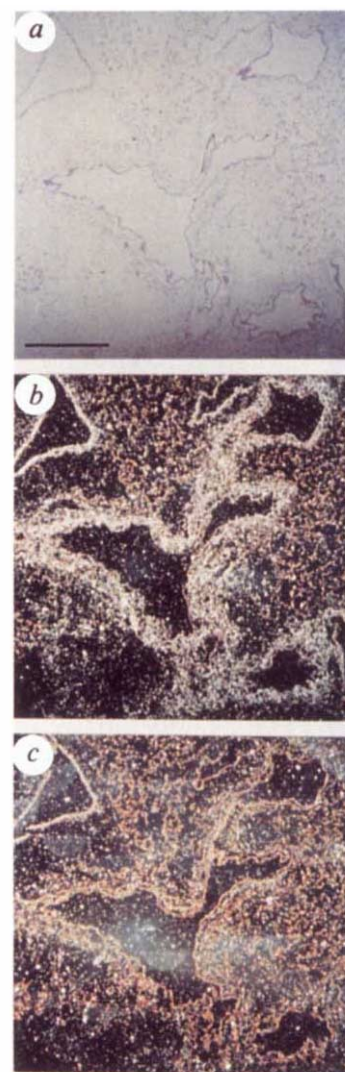
Decreasing gradients of expression of CFTR along both the crypt–villus and proximal–distal axes of the intestine (Fig. 3) may be indicative of the importance of CFTR in the overall functioning of this tissue: the expression of several genes, including those for transforming growth factor type- β , sucrase–isomaltase and cryptdin, all important in intestinal function, is also regulated on one or both axes^{12–14}. It has been shown that intestinal crypt cells are responsible for Cl[−] secretion⁸. Cyclic AMP-evoked Cl[−] conductances are maximal in undifferentiated Caco-2 and HT-29 cells, which resemble crypt cells, and the regulated anion conductance is mediated by a small, linear channel (C. Bear, personal communication) similar to that observed after transfection of CFTR cDNA (refs 15–18). Secretion of Cl[−] by ileal and rectal crypt cells is defective in CF, whereas the absorption of Cl[−] by the villus cells is unaffected^{19,20}. Expression of CFTR in the intestinal crypts is consistent with CFTR being responsible for Cl[−] secretion. Minimal CFTR expression in the villi implies that a Cl[−] absorption mechanism exists other than that in the ducts of the sweat and salivary glands, where it seems to be mediated by CFTR^{4,9}. In the villus epithelium of the guinea-pig small intestine, Cl[−] transport is mediated by an outwardly rectifying channel that is not stimulated by cAMP (ref. 21).

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CFTR is expressed in the epithelial lining of the uterus (Fig. 4 *a-c*) and this may explain the reduced fertility of women with CF. The stage-specific expression of *CFTR* in the testis (Fig. 4, *d-f*) suggests a function for *CFTR* in spermatogenesis. Other genes whose products are thought to be important in spermatogenesis, such as protamines in the compaction of spermatid DNA^{22,23} and possibly hemiferrin in iron metabolism of germ cells²⁴, also have their expression regulated during the cycle of the seminiferous epithelium. Obstruction or absence of the *vas deferens*, characteristic of men with cystic fibrosis, is one of the primary causes of their infertility²⁵. But there are disturbances of spermatogenesis resulting in dysmorphic spermatozoa^{5,7}. These defects, considered in light of the stage-specific expression of *CFTR* in the testis, indicate either a critical requirement for a regulated Cl^- channel during normal spermatogenesis or the involvement of *CFTR* in the transport of an alternative substrate important for sperm maturation, or a combination of the two.

FIG. 2 Lung tissue hybridized with ³⁵S-labelled *CFTR* RNA and stained with haematoxylin. *a*, ▶ Bright-field view of sections hybridized to antisense strand; *b*, dark field, antisense strand; *c*, dark field, sense strand. *CFTR* expression is observed as a broad band of hybridization that encompasses both the epithelial lining and the underlying lamina propria of the bronchi and bronchioles. There are a variety of cell types present within the lamina propria, and at higher magnification *CFTR* expression does not seem to be localized to any individual cell types (not shown). Rather, there is an even distribution of silver grains over the entire structure. The sections of lung tissue include both large and small airways, but submucosal glands are not present in these sections as in rats the glands are found only in the trachea²⁹. *CFTR* is expressed at much lower levels in the lung than in some of the other tissues examined, making pre-hybridization digestion of sections with proteinase K essential to observe positive hybridization signals. Proteinase K digestion also results in diminished tissue preservation. Scale bar, 400 μm .



Collectively, our results demonstrate an exquisite regulation of *CFTR* expression which, with the disease pathology, leads us to conclude that the role of *CFTR*, at least in the salivary glands, the pancreas and the intestine, is to transport Cl^- ions. The observation that *CFTR* is specifically expressed in secretory intestinal and reabsorptive salivary duct cells is consistent with *CFTR* being responsible for the bidirectional transport of Cl^- *in vivo*, suggesting that in these tissues *CFTR* functions as a

regulated Cl^- channel. In tissues such as the lung or testes, where expression is not restricted to surface epithelia, *CFTR* may perform other functions. There it might be an active transporter, as predicted from the sequence and proposed domain organization of the protein^{2,26,27}. The broad expression of *CFTR* in lung may contribute to the complex pathology in airways of CF patients⁵. The stage-specific expression of *CFTR* in the testis could explain abnormalities in spermatozoa of CF patients and could preclude *in vitro* fertilization as an option for many patients⁷. The unexpected presence of *CFTR* mRNA in the lamina propria of the lung and the seminiferous tubules of the testis demonstrates how analysis of *in vivo* cell-specific

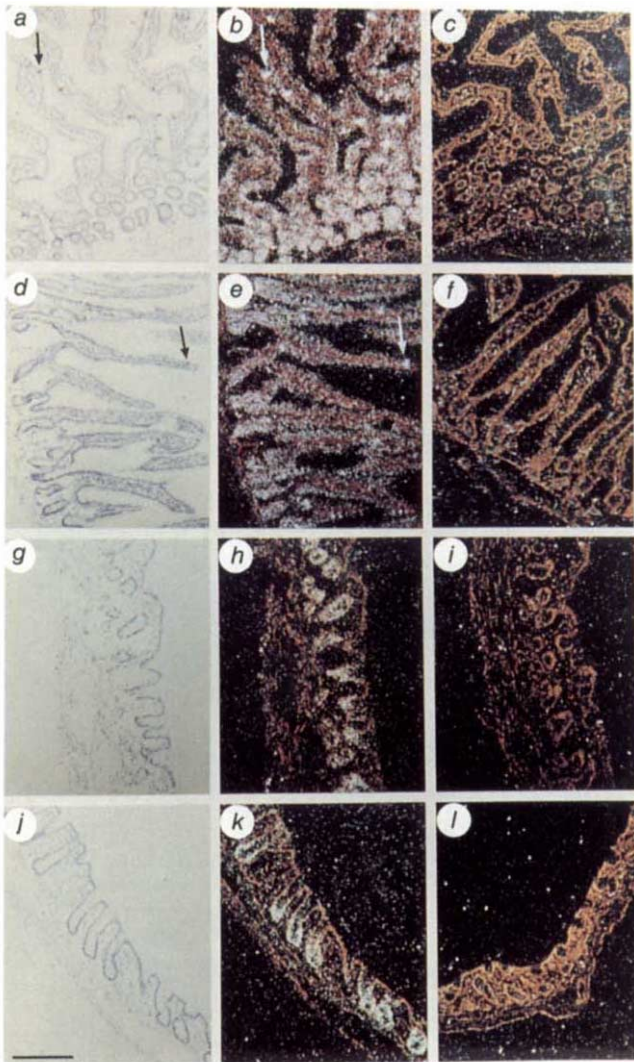


FIG. 3 Duodenum (a–c), jejunum (d–f), proximal colon (g–i) and distal colon (j–l) hybridized with ^{35}S -labelled *CFTR* RNA and stained with haematoxylin. a, d, g and j, Bright-field view, antisense strand hybridization; b, e, h and k, dark field, antisense strand; c, f, i and l, dark field sense strand. The principal site of *CFTR* expression in the intestine is the crypt of Lieberkuhn (a, b, d and e) and the levels of *CFTR* mRNA decrease gradually as the cells differentiate and migrate from the crypt to the villus. Fully differentiated cells at the villus tip contain little *CFTR* mRNA. In the colon, the gradient on the vertical axis shows a distinct boundary: *CFTR* expression ceases ~2/3 of the distance between the crypt and the surface epithelium (g, h, j and k). The gradient of *CFTR* expression along the length of the intestine is maximal in the duodenal crypts and gradually declines towards the distal colon. Individual cells that express *CFTR* mRNA in very high amounts can occasionally be seen on the edge of the villi of the small intestine, appearing as black spots in bright field (a and d, arrowed) and as the corresponding white spots in dark field (b and e, arrowed). Higher magnification reveals two cell populations, one epithelial in origin and the other lymphoid and derived from the lamina propria (not shown). Scale bar, 200 μm .

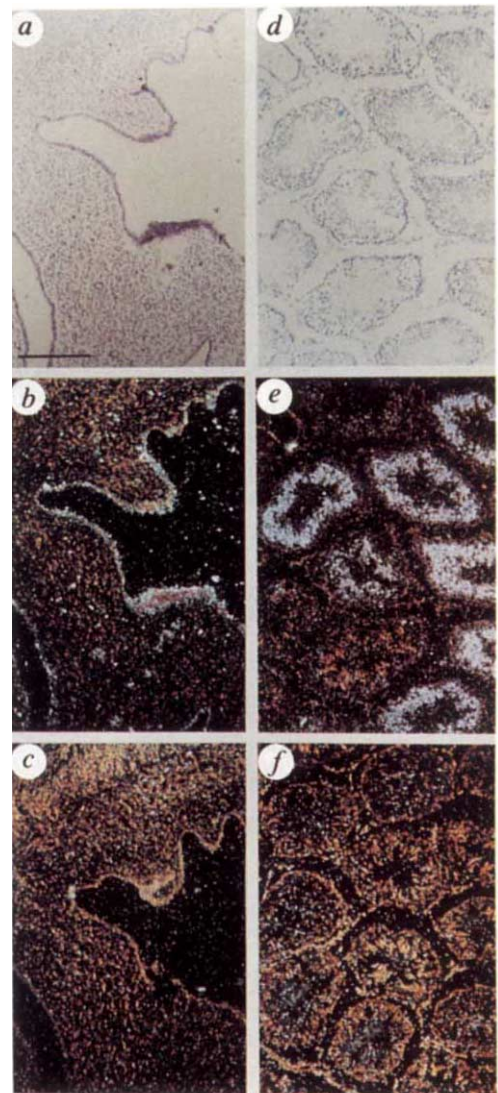


FIG. 4 Uterus (a, b and c) and testis (d, e and f) hybridized with ^{35}S -labelled *CFTR* RNA and stained with haematoxylin. a and d, bright-field view, hybridization to antisense strand; b and e, dark field, antisense strand; c and f, dark field, sense strand. *CFTR* mRNA is detected only in the epithelial lining of the endometrium of the uterus (a and b) with no hybridization in the underlying endometrial stroma, the myometrium or the perimetrium. *CFTR* mRNA is undetectable in lactating mammary gland and is only detectable by polymerase chain reaction (PCR) in the ovary (not shown). In the testis (d and e) the level of *CFTR* mRNA in individual tubules is highly variable, reflecting the stage-specific expression of *CFTR* with respect to the cycle of the seminiferous epithelium. On the basis of tissue morphology³⁰, *CFTR* is maximally expressed in the round spermatids of stages VII and VIII, with little expression in germ cells at other stages of maturation. *CFTR* does not seem to be expressed in Sertoli and Leydig cells, the somatic cells of the testis. Scale bar, 200 μm .

expression of *CFTR* can provide a new perspective from which to consider the pathology and treatment of the disease, as well as the normal function of *CFTR*. □

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A product of the *prune* locus of *Drosophila* is similar to mammalian GTPase-activating protein

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THE X-linked *prune* (*pn*) eye-colour mutation of *Drosophila melanogaster* has a highly specific, complementary lethal interaction with the conditional dominant *Killer of prune* (*awd^{K-pn}*) mutation^{1,2}. Although *awd^{K-pn}* flies have no apparent phenotype on their own, *pn awd^{K-pn}* double mutants die as second or third larval instars. The *awd* locus encodes a nucleoside diphosphate kinase³, an enzyme that catalyses the transfer of high-energy phosphate bonds between nucleoside diphosphates and nucleoside triphosphates⁴, which is essential for the normal development of *Drosophila*. Analysis of the *pn* locus has suggested that the complementary DNA, TcD37, encodes a putative *pn⁺* product⁵. Here we report the nucleotide sequence of TcD37 and the similarity of its deduced protein product to the catalytic domain of mammalian GTPase-activating proteins (GAPs); GAPs stimulate the GTPase activity of Ras (ref. 6), which are plasma membrane-bound proteins involved in the regulation of cell proliferation and differentiation⁷. These results suggest that the *Drosophila* TcD37 protein participates in a biochemical pathway similar to that of Ras and GAPs in mammals and yeast. We propose that the interaction between *pn* and *awd* is due to a neomorphic mutation that enhances the ability of *awd^{K-pn}* nucleoside diphosphate kinase to induce a regulatory GTPase into a GTP-bound 'on' state, whereas *pn* modulates the activity of this GTPase either by switching it to a GDP-bound 'off' state or by interfering with its effector function.

The brown eye-colour phenotype of *pn* flies is due to an alteration in the activity of GTP cyclohydrolase^{8,9}, the enzyme that converts GTP to dihydroneopterin triphosphate in the first step of pteridine biosynthesis¹⁰. Gene dosage experiments indicate that *pn* may be one of several genes involved in the developmental regulation of GTP cyclohydrolase^{8,9}, whereas the structural gene of the enzyme is encoded by the *Punch* (*Pu*) locus¹¹. *Killer of prune* belongs to the lethal complementation group, *abnormal wing discs* (*awd*, ref. 2). During the third larval instar stage, the wing, leg and eye-antenna imaginal discs of homozygous *awd* null mutants differentiate with varying degrees of

aberration and cell death¹². There are also abnormalities in *awd* ovaries, larval brain and proventriculus, and these homozygotes eventually die as prepupae. The *Awd* protein is a nucleoside diphosphate (NDP) kinase with a proposed role in microtubule polymerization, and it is 78% identical to Nm23, a human cognate involved in metastatic tumour suppression^{3,13}. The

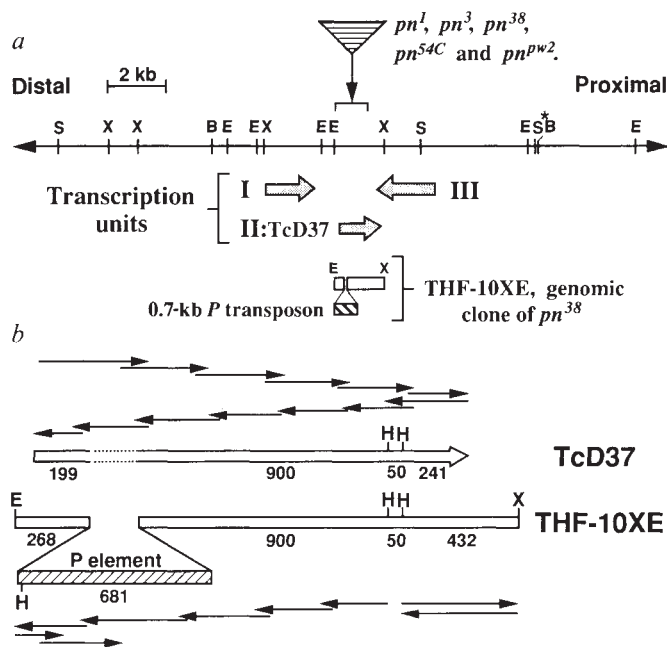


FIG. 1. *a*, Molecular map of the *pn* locus. The lesions of *pn¹*, *pn³*, *pn³⁸*, *pn^{54C}* and *pn^{pw2}* are located in the 1.2-kb genomic fragment indicated. Three transcription units, I, II and III, have been identified the central portion of the genomic region shown. TcD37 is the cDNA of transcription unit II, whereas THF-10XE is a genomic clone isolated from the *P* allele, *pn³⁸*. The directions of the distal and proximal tips of the X chromosome are indicated. Restriction sites: B, *Bam*HI; E, *Eco*RI; S, *Sal*I; and X, *Xho*I. Also shown are the *Hind*III (H) sites present in TcD37 and THF-10XE, and the sizes (base pairs) of corresponding DNA fragments. *b*, Strategy for determining the complete and partial nucleotide sequences of TcD37 and THF-10XE, respectively. Regions that were sequenced are depicted by arrows.

METHODS. The genomic and cDNA fragments were inserted into the polylinker region of pBluescript II KS phagemid (Stratagene) and used to transform DH5- α F' cells (Bethesda Research Laboratories). Nested deletions of recombinant phagemid were made according to the method described by Henikoff³¹. Single-stranded DNA was isolated from transformed DH5- α F' cells according to the procedure described by Promega Corporation and subsequently sequenced by the chain termination method³² following the Sequenase 2.0 Version protocol (USB); α -³⁵S-labelled dATP (Du Pont), T3 and T7 primers were used in the reactions.

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nucleotide sequence of *awd*^{K-pn} has revealed that *Killer of prune* is a missense mutation of *awd*⁺ that results in the single amino-acid substitution of serine for proline at residue 97 (A. Shearn, personal communication). The neomorphic function of *awd*^{K-pn} that causes lethality in *pn awd*^{K-pn/+} mutants, however, is still a mystery.

As a step towards understanding the *pn awd*^{K-pn} lethal phenomenon, we isolated the *pn* locus from the *P* allele, *pn*³⁸ *bw st*, by transposon tagging⁵; the molecular organization of the genomic region is shown in Fig. 1a. Previous studies identified at least three transcription units (I, II and III) in this genomic region and suggested that transcription unit II encodes a putative *pn*⁺ product⁵. Following the strategy depicted in Fig. 1b, we sequenced the cDNA corresponding to transcription unit II, TcD37, as well as its *P* element-containing genomic homologue from *pn*³⁸ *bw st*, THF-10XE (Fig. 2). The nucleotide sequences show that the 1.4-kilobase (kb) cDNA consists of a single exon and reveals the presence of a putative TATA box, transcription initiation site and polyadenylation signal. This information suggests that TcD37 is a full-length cDNA and that transcription proceeds from left to right (Fig. 1); this direction of transcription has been confirmed by northern analysis using antisense RNA probes⁵. As with many eukaryotic genes, the longest open reading frame follows the first ATG of TcD37, and a purine and G residue are present at the -3 and +4 positions, respectively, relative to this ATG start codon¹⁴. If this site is used for the initiation of translation, the transcript will have a 70-base pair (bp) untranslated leader sequence and will generate a 365 amino-acid polypeptide of relative molecular mass 41,000 (*M_r*, 41K) with two putative *N*-glycosylation sites and two potential sites of phosphorylation by tyrosine kinase (Fig. 2).

To examine the function of the TcD37 product, we searched for similarities of the deduced protein to characterized gene products. When the NBRF Protein databank (Release 21.0) was examined using WORDSEARCH (GCG Sequence Analysis Software Package, version 7, Genetics Computer Group, 1991), the predicted TcD37 polypeptide showed similarity to the carboxy-terminal portion of bovine GAP (Fig. 3a), a 1,044 amino-acid protein that attenuates the activity of Ras by stimulating the hydrolysis of Ras-bound GTP to GDP. The catalytic domain of GAP, necessary and sufficient to activate the GTPase of Ras, has been localized to 350 residues at the C terminus of GAP (ref. 15). The similarity of the entire TcD37 protein to GAP was almost completely in this functional domain.

In addition to bovine GAP, the genes of several other proteins with GAP activity have been characterized. These include the GAPa and GAPb human cognates¹⁶, human neurofibromatosis type-1 (Nf1) protein¹⁷⁻²⁰, and yeast Ira1 and Ira2 (refs 21, 22; Fig. 3b). We did pairwise comparisons of the deduced amino-acid sequence of TcD37 with the functionally related catalytic domains of hGAPa, Nf1 and Ira1 (Fig. 3c). The identities between hGAPa and Nf1, Ira1 and Nf1, as well as hGAPa and Ira1 were 27%, 28% and 21%, respectively, similar to homologies previously reported¹⁷. In this analysis, the entire TcD37 polypeptide was 20%, 19% and 16% identical to hGAPa, Nf1 and Ira1, respectively. Recently, the most conserved sequences among the catalytic domains of these proteins were aligned and compared to JC99 and JC265, two human cDNAs that interfere with the function of Ras in yeast²³. The deduced TcD37 protein, along with HP3-12 and HuB10-A (two bovine rap1GAPs which specifically stimulate GTP hydrolysis by the *rap1/Krev-1* GTPase²⁴), have sequences that are similar to this conserved region (Fig. 3d). Overall, the homologies for TcD37 protein

FIG. 2 The nucleotide and deduced amino-acid sequences of TcD37. The DNA sequence 5' of TcD37, from positions -129 to -71, was obtained from THF-10XE. A proposed heptanucleotide ATCTGGC transcription initiation site, present at position -70 of TcD37, is similar in five of the seven bases to the reported insect consensus for non-heat shock genes, ATCA(G/T)(C/T)³³. A possible TATA box is located at position -96 and the common polyadenylation signal AATAAA is found in the 3' region of the cDNA. The initiation and termination codons of translation are underlined. The 5'-GGTGGGCC-3' site (position 138) into which the 681-bp *P* transposon of THF-10XE inserted, is complementary to 5'-GGCCACC-3', which corresponds in five of the eight bases with the reported consensus of GGCCAGAC for *P* insertion sites³⁴. The +++ symbol indicates the attachment site of the poly(A)⁺ tail. The asterisks mark two putative *N*-glycosylation sites at asparagine residues of positions 30 and 41, as well as two putative phosphorylation sites by tyrosine kinase at residues 30 and 41. A Kyte and Doolittle hydropathicity analysis³⁵ of the deduced amino-acid sequence of TcD37 showed no evidence of an obvious transmembrane-spanning domain (data not shown).

-129	TGAAGAGCACCAGAAGTGAGAAGGTCAAGCACTAATAG	-91
-90	GGTTTCTCTTCTCGCGCAGCATCTGGCGGAGGCTCACCAGTTCGCTGGGCTGCTGCTCCGACGTTTCCGGCCGAAATTACATCTGGTA	-1
+1	ATGGGCAACGAATCGTGACTTGGACTCCGCCGCTTTCGGCCGTCACCTTGGCTTTGTCTACGCGCAGCGTCATCGGAGCAGCACTAT	90
1	MetGlyAsnGluSerCysAspLeuAspSerAlaValSerAlaValThrLeuAlaPheValTyrAlaGlnArgHisArgGluHisAspTyr	30
91	GTACCTATACTGAACATTCCTCGCCGGGACTACCGGTGAAACCGAGCTGGGCGCACTTGTGTGAAATGTGGGATTCGGCAGCCCGTG	180
31	ValProIleLeuAsnIleProArgArgAspTyrProLeuLysThrGluValGlyHisLeuPheValLysCysGlyIleAlaGluProVal	60
181	TGTGCTTCCGAGACGATATTCGCCGGAGGTGGTCCAGGATGTGAACGTTATCTCGTGACCACCATGTAAGCCCGCTGGCGCAAAAT	270
61	LeuLeuPheArgAspAspIleProArgGluValValGlnAspValAsnValIleLeuValAspHisHisValSerProLeuAlaProAsn	90
271	GTTACTGAAATTTTGGATCACAGGCCCTTGGAGGACAGCAGTCCATCCTTCAAGCAGCTGCCAACCTTGCCAACTGGACATAGATGCC	360
91	ValThrGluIleLeuAspHisArgProLeuGluAspSerSerProSerPheLysGlnLeuProThrLeuCysGlnLeuAspIleAspAla	120
361	TCGGTGGGTTCTCGCCCACTCTGGTGGCCAGCGGTATTGGCAGAGGACCAACCCGATCCATAGCTAGCGTGGCCAGCTGCTGCACGCC	450
121	SerValGlySerCysAlaThrLeuValAlaGlnArgTyrLeuAlaGluAspGlnProArgSerThrSerValAlaGlnLeuLeuHisAla	150
451	ACCATCGTCTGGACACAAATTAATTTGCACCCGCGGCCAAGCGCTACGGGCCAAGGACGAGCCATCGTACAGAAAGTTGGAGAGCGAG	540
151	ThrIleValLeuAspThrIleAsnPheAlaProAlaAlaLysArgTyrGlyProLysAspGluAlaMetValGlnLysLeuGluSerGlu	180
541	CTTAACCGTAAGGACGCTCAAGAAGTAGCCTTTTGTATGAGCTAGTGGCTGCAAGGCGGATATAGTAAGCTAATCTTCCCGAAGTT	630
181	LeuAsnArgLysAspAlaGlnArgSerSerLeuPheAspGluLeuValAlaAlaArgAlaAspIleSerLysLeuThrLeuThrGluVal	210
631	TTGGCGCAAGGATATGAAGTCTTGCAACCGATCGTCAGGTGGTTCCTTCTAGCTGGAATGCCATCCTAGTCAGAGATTTTGTGGAGAAA	720
211	LeuArgLysAspMetLysValLeuThrAspArgGlnValValProLeuAlaGlyMetProIleLeuValArgAspPheValGluLys	240
721	AGCGGCGCCGAAAAAGCGTTCGCGAGTTTGGCGTGGAGAGTAACCTTTTGGTTATCTCGGAATGTATGTACCTGCGCATGGCCAG	810
241	SerGlyAlaGluLysAlaValArgGluPheGlyValGluSerAsnLeuLeuValIleLeuGlyMetTyrValSerProAlaAspGlyGln	270
811	GTGCAGCGTGACCTGGCGTGTATCTCTCTCCGCCCAAGGCCAATTCGTTCAACGCTCCAGCAAGCAGTCTGAGTCTAAGCATCCA	900
271	ValGlnArgAspLeuAlaLeuIleSerLeuSerGlyGlnGlyGlnPheValGlnArgValGlnGlnAlaLeuMetGluSerAsnAspPro	300
901	AAATGGAGTTGGACCTCAGAGGTGGACACCCGCTTATGGCGGCTGCTTCTTGGCCCAACACACCTCCAGGCCACCGAAGCAC	990
301	LysLeuGluLeuArgProHisGluValAspThrArgPheMetGlyGlyCysPheLeuArgGlnHisAsnValGlnAlaThrArgLysHis	330
991	ATCTGCCCCATTTGTAAGCGAGCGCTGCTTGAATGGGAAGCGGATCAGCCCTCGGATTTGTACAGAGGTGTACTTCTTCAAGGAGAGCCG	1080
331	IleLeuProIleValLysArgAlaLeuLeuTyrGluAlaAspHisAlaCysAspCysAspGluValTyrPhePheLysGluLysPro	360
1081	CAGCTGGGACTCTCTTAAGAGAGATCAGGCCAAACCGAAGCTTAGACAAGACTTAGCTATAATTTAGCGCAACCGTTATGTTACCAAC	1170
361	GlnLeuGlyLeuSerEnd	365
1171	GTTAAGCTTACACCAAAAGACATAGCGAATTTCCCATTTTACGATAAATGACGAAGGCAAGCGAGTAAATGGAAGCAATCAGTATT	1260
1261	CCAAATTTAAGTTACTTTGCAACCTTGGTAACATAAAGGCAAAATAGTTTTCAGAGTATTCACACATTAAGCTCTATCCAGTTTCTAGTT	1350
1351	GTATTATATGGATTAAACGATTATGTACAGGTATACATGGGTAAGAAAGCGTTATGATTAAATAAAAGGACTACAA+++	1431

might suggest that it acts in a biochemical pathway involving a GTPase related to Ras. This would not be unexpected as Ras belongs to a superfamily of GTPases and the activities of these enzymes seem to be modulated by several protein factors, each of which may be specific for a given GTPase^{7,25}. For example, in addition to rasGAP and rap1GAPs, GAPs specific for the Rho, RalA and Rab3A GTPases have been identified²⁶⁻²⁸.

The possibility that the *pn* locus may encode a GAP-like protein provides a novel explanation for the *pn awd^{K-pn}* interaction. When this lethal phenomenon was first discovered, Sturtevant proposed that the loss of Pn results in the production or accumulation of a substance that Awd^{K-pn} converts into a toxic product¹ (Fig. 4a). We propose alternative models (Fig. 4b). It has been suggested that NDP kinases are involved in regulating the activation of some GTPases, such as Ras and G proteins, through direct protein interactions and high-energy phosphotransfer reactions^{29,30}. In addition to the proposed role of Awd NDP kinase in microtubule polymerization, it may

regulate the activity of a Ras-like GTPase. A missense mutation might confer a neomorphic function that enhances the ability of Awd^{K-pn} to activate this quiescent GTPase, or to maintain its GTP-bound form longer than it normally should. In an *awd^{K-pn}* mutant, TcD37 protein may function as a GAP-like negative regulator which stimulates GTP hydrolysis by the GTPase. Alternatively, TcD37 protein may act like the products of JC99 and JC265 by interfering with GTPase effector function (Fig. 4b, iii), possibly by acting as a competitive antagonist. By contrast, in the *pn awd^{K-pn}/+* double mutant, the absence or severe reduction of TcD37 protein produces a higher amount of activated GTPase and this eventually causes lethality. These models predict that a second site suppressor mutation of the *pn awd^{K-pn}* lethality will be in such a GTPase or a protein factor that modulates its activity, or possibly a downstream effector in this biochemical pathway.

The diverse roles of regulatory GTPases, in processes such as protein synthesis and translocation, vesicular transport, signal

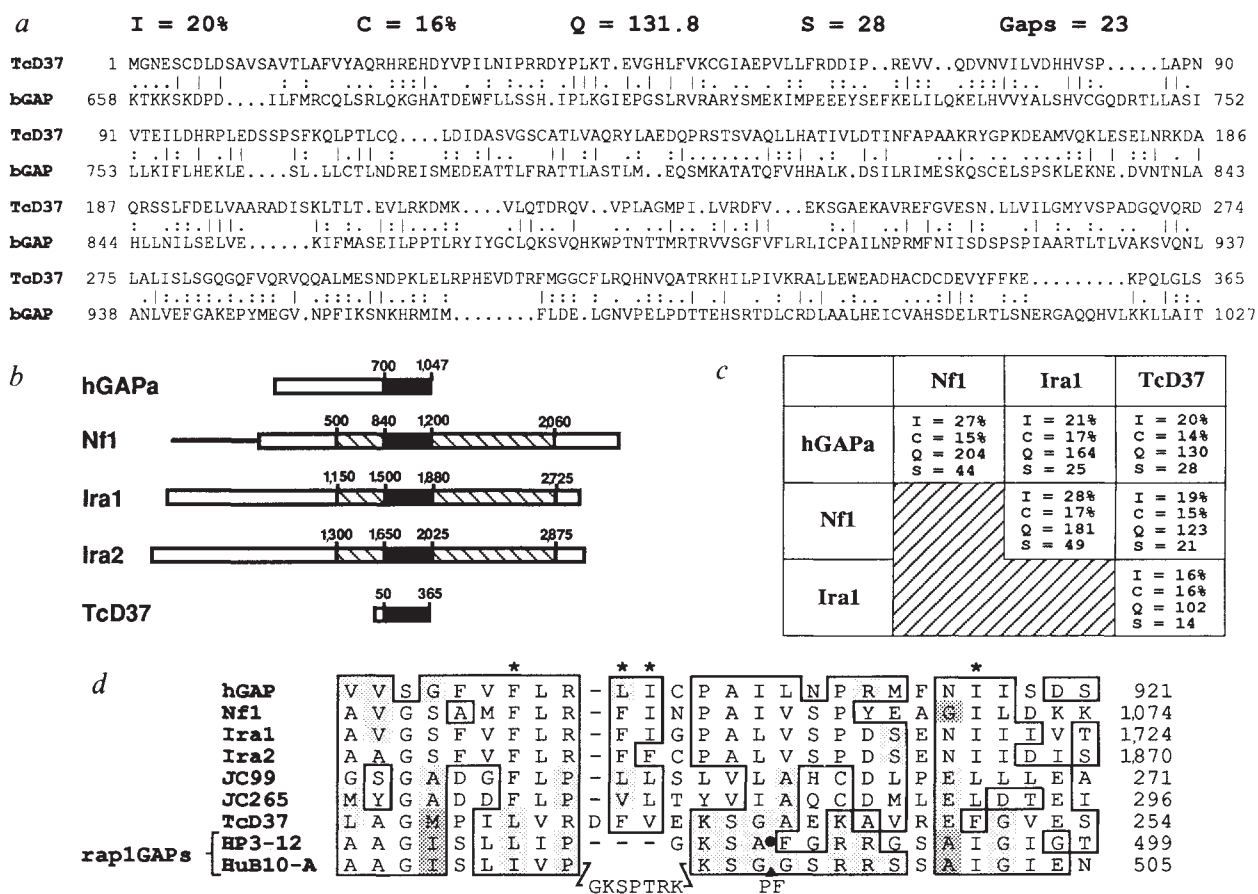
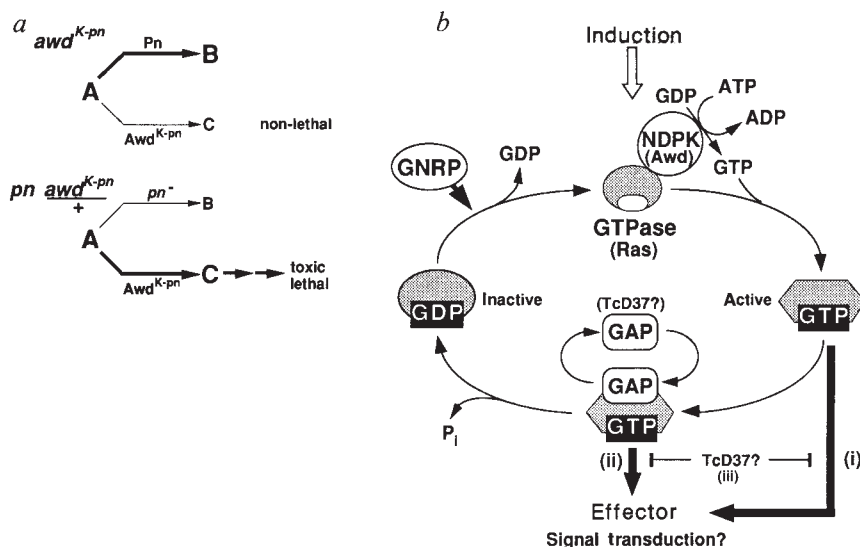


FIG. 3 Similarities among the deduced TcD37 product and the catalytic domains of GAP and GAP-like proteins (single-letter amino-acid code). **a**, The optimal alignment of TcD37 protein with bovine GAP (bGAP) using WORDSEARCH (GAP weight=2.00; GAP length=0.10). The results shown are the following: I, identical amino acids; C, conserved substitutions; Q, Quality; and S, Score. Conservative substitutions were determined according to these groupings: MILV; FYW; ST; AG; DE; NQ; RK. To determine the statistical significance of this pairwise comparison, the deduced amino-acid sequence of TcD37 was randomized independently 25 times and realigned with the catalytic domain of bGAP. Analysis of the qualities obtained for these comparisons showed that the probability of attaining a Q value of 131.8 or higher for the bGAP catalytic domain with a random sequence of this amino-acid composition is less than 0.05. **b**, Regions of homology among the predicted proteins of human GAPa (hGAPa), Nf1, Ira1, Ira2 and TcD37. The solid boxes represent the catalytic domains of hGAPa, Nf1, Ira1 and

Ira2, whereas the corresponding region of TcD37 shows similarity to these functional domains. Ira1 and Ira2 have additional homology to Nf1 in the sequences flanking their catalytic domains^{17,18} (hatched boxes). **c**, Summary of the WORDSEARCH pairwise comparisons among the solid boxed regions of hGAPa, Nf1, Ira1 and TcD37. **d**, A comparison of the most conserved region in the catalytic domains of GAP and GAP-like proteins. The amino-acid position in each sequence is shown on the right. Similar residues are boxed and shaded areas represent a different group of conserved residues in a given column. It appears that F residues may substitute for L, I or V residues (or vice versa) at critical positions marked by asterisks. Spacing was altered to accommodate the alignments of TcD37 and rap1GAPs. HP3-12 and HuB10-A are two versions of rap1GAP (ref. 24); compared with HuB10-A, HP3-12 has a 26 amino-acid duplication of this region. The filled circle between the A and F residues of HP3-12 rap1GAP marks the position where residues SR should be (not shown), while HuB10-A has PF instead.

FIG. 4 *a*, Model proposed by Sturtevant to explain the lethality of *pn awd^{K-pn}* double mutants. In an *awd^{K-pn}* mutant, Pn converts product A to B while Awd^{K-pn} competes for A to generate C. But the higher affinity of wild-type Pn for A results in the production of non-lethal doses of C by Awd^{K-pn}. In the *pn awd^{K-pn}* double mutant, the absence or severe reduction of the *pn⁺* product causes A to accumulate, thus allowing Awd^{K-pn} to generate lethal quantities of C. *b*, The biochemical cycle of a Ras-like GTPase and its interacting proteins. In this schematic, the GTPase acts as a molecular switch in a signal transduction pathway, similar to the role of heterotrimeric G proteins. In such a pathway, the transduction of an extracellular signal would prompt the GTPase to bind GTP and activate an effector protein, either through its GTP-bound form (i) or as a GAP-GTPase-GTP complex (ii). These two possibilities are due to evidence indicating that in addition to its role as a negative regulator, GAP seems to act as a positive effector in some systems⁷. The activity of GTPase is modulated by GAPs and guanine nucleotide releasing proteins (GNRPs)²⁵. Stimulation of GTP hydrolysis by GAP yields inactive GTPase-GDP, which the GNRPs act upon to induce the liberation of GDP. It has been proposed that NDP kinase (NDPK) generates the GTP that activates the effector function of some GTPases^{29,30}; it is also plausible that NDPK directly phosphorylates



GTPase-GDP to yield GTPase-GTP. The possible roles of the TcD37 product as a GAP-like negative regulator or as a protein that interferes with GTPase effector function (iii) are also shown.

transduction, cell proliferation and differentiation, are being continually uncovered. These GTPases work dynamically alongside a growing family of GAPs and our findings may support the notion that NDP kinases modulate the activities of some of these GTPases. The role that a GAP-like protein may have in controlling pteridine biosynthesis in *Drosophila* is not yet obvious, but the characterization of the *pn* and *Pu* loci provide a framework for elucidating the mechanisms by which these genes interact. □

Rapid spread of an inherited incompatibility factor in California *Drosophila*

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IN *Drosophila simulans* in California, an inherited cytoplasmic incompatibility factor reduces egg hatch when infected males mate with uninfected females¹⁻⁷. The infection is spreading at a rate of more than 100 km per year; populations in which the infection was rare have become almost completely infected within three years. Analyses of the spread using estimates of selection in the field suggest dispersal distances far higher than those found by direct observation of flies. Hence, occasional long-distance dispersal, possibly coupled with local extinction and recolonization, may be important to the dynamics. Incompatibility factors that can readily spread through natural populations may be useful for population manipulation and important as a post-mating isolating mechanism.

Parasites will tend to spread if parasitized hosts have a reproductive advantage. This occurs in the parasite-mediated 'cytoplasmic incompatibility'^{8,9} identified among several orders of insects¹⁰⁻¹⁶. We discovered a unidirectional incompatibility system in California *Drosophila simulans*¹. Females from central or northern California produced relatively few progeny when mated to males from the Los Angeles basin of southern California, whereas the reciprocal cross and crosses within localities produced normal numbers of progeny. The incompatibility is apparently caused by an intracellular, Wolbachia-like parasite which can be eliminated by antibiotics^{1,7} and is detectable by electron microscopy^{5,6} and fluorescent stains⁷.

The infection is generally transmitted maternally; in the laboratory, infected females produce infected male and female offspring regardless of the type of male mated, whereas paternal transmission is rare². There is no evidence of frequent horizontal

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transmission (our unpublished results). Although the microorganisms are not found in mature sperm⁴, their presence seems to modify sperm so that they are no longer compatible with an uninfected female's eggs, causing many embryos to die. This effect allows the parasite to spread rapidly, because infected females can mate with any male and produce many progeny, but uninfected females produce relatively few progeny when they mate with infected males. The parasite decreases slightly the number of eggs that host females produce but has no other major ill effects³.

We have monitored Californian populations since 1984 and there has been a rapid spread of the infected type, denoted R. Our 1984 samples pooled at least 20 isofemale lines from each site. They indicated that the R-type was restricted to southern California. But after 6 years, the infection is now pervasive in Watsonville (the collection site of our uninfected reference stock) and Ivanhoe, over 240 km from Los Angeles (Fig. 1). Moreover, the infection frequency is now nearly 10% at Healdsburg and Gridley, more than 600 km north of Los Angeles. Repeated sampling indicates rapid increases at Arvin and Sanger/Ivanhoe, while the infection frequency has remained at ~95% at Riverside/Highgrove (Fig. 2). The persistence of rare uninfected individuals in southern California can be explained by infrequent production of uninfected progeny by infected mothers³.

We can interpret our data with simple models of frequency change. Let p denote the frequency of infected adults, let $q = 1 - p$, let $H \equiv 1 - s_h < 1$ denote the relative hatch rate for eggs from incompatible crosses, and let $F \equiv 1 - s_f \leq 1$ denote the relative fecundity of infected females. Thus s_h is the relative reduction in egg hatch caused by incompatible crosses, and s_f is the relative reduction of female fecundity caused by the infection. Assuming random mating and discrete generations, the infection frequency in an isolated population changes each generation according to

$$\Delta p = \frac{s_h p q (p - \hat{p})}{1 - s_f p - s_h p q} \quad (1)$$

where $\hat{p} = s_f / s_h$ is an unstable equilibrium frequency above which the infection will sweep through the population and below which it will be eliminated^{3,8,9}. Information from field-collected females suggests that $s_h \approx 0.45$ and $s_f \approx 0.05$, so that $\hat{p} \approx 0.11$ (ref. 3). Modifying equation (1) to include the effects of migration in a population continuously distributed in one dimension¹⁷⁻¹⁹, if $\hat{p} < 0.5$, then the infection will spread after it becomes established in one place. It is expected to move eventually as a travelling wave approximately described by

$$p(x, t) = \frac{1}{2} \left[1 + \tanh \left(\frac{x + vt}{2w} \right) \right] \quad (2)$$

where $p(x, t)$ is the infection frequency at point x at time t , $v = (\sigma \alpha \sqrt{s_h})/2$ is the wave speed, $\alpha = 1 - 2\hat{p}$, σ is a measure of the average migration distance (the standard deviation of the distance between the birthplace of parents and offspring), and $w = \sigma/\sqrt{s_h}$ describes the width of the wave, such that at any time the frequency of the infection is expected to go from $p \approx 0.05$ to $p \approx 0.95$ over $\Delta x = 3w$. Substituting our selection parameter estimates, we expect the parasite to spread throughout California at a rate of $\sim 0.26\sigma$ per generation with $w \approx 1.49\sigma$. The width, w , can be estimated from data along a transect, because equation (2) implies that at any fixed time, $\ln \{p(x, t)/[1 - p(x, t)]\}$ changes linearly with x with slope w^{-1} .

According to equation (1) with $s_h = 0.45$ and $s_f = 0.05$, once the frequency of the R-type is near 0.2, it will increase to near 0.8 within 20 generations. Over this period, the contribution of migration to local frequency change is expected to be small. At both Arvin and Sanger/Ivanhoe, which are ~ 160 km apart in the southern Central Valley, the frequency of the infection increased from roughly 0.3 to above 0.8 in about one year.

Iterating equation (1), we see that this is consistent with our selection parameter estimates if these populations produce about 15 generations per year. This is plausible, given that *D. simulans* can usually be caught throughout the Central Valley from late April until November, and its sibling species *D. melanogaster* can produce adult offspring within 11 days in nature²⁰.

The frequency differences in the 1990 samples from central and northern California provide estimates of w and σ . There are statistically significant differences between our collections at Watsonville and Healdsburg, Ivanhoe and Escalon, and Escalon and Davis, which are separated by approximately 410, 175 and 205 km, respectively. Using equation (2), we estimate σ as approximately 57, 50, and 60 km per gen^{1/2} from each pair, respectively. Using the extreme values from the confidence intervals for the infection frequencies (Fig. 1) reduces these estimates

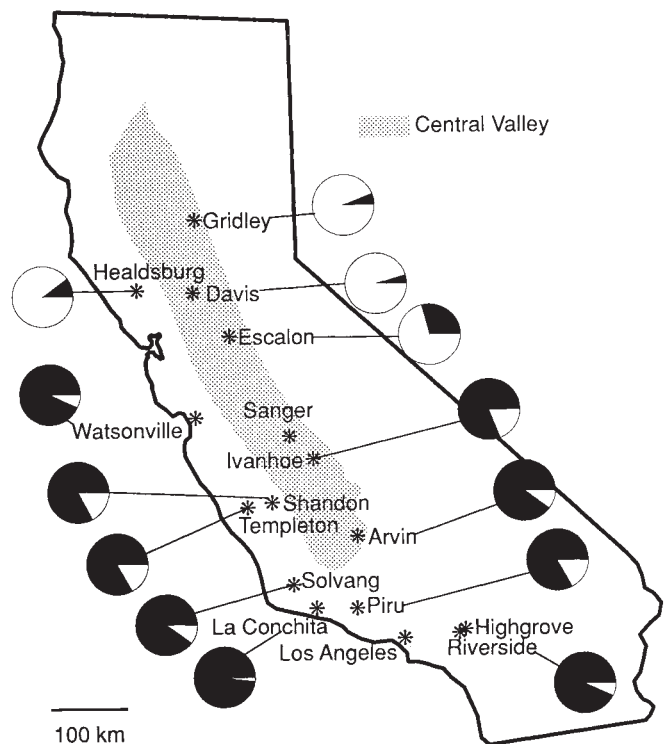
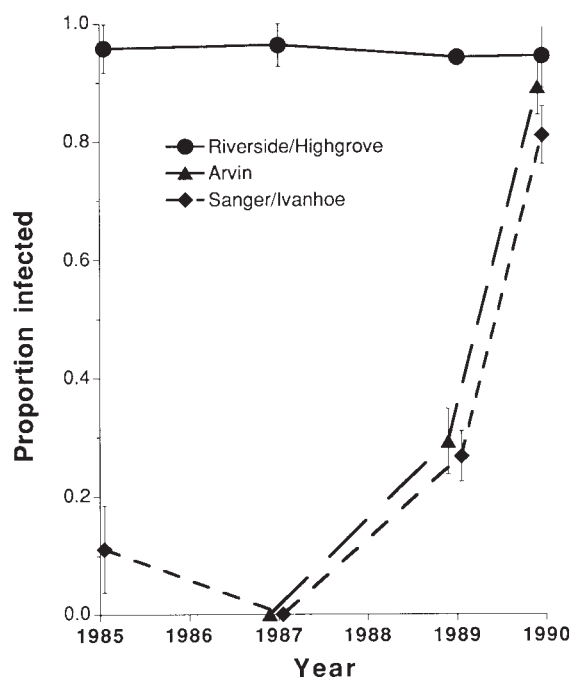


FIG. 1. Approximate location of California collection sites in October 1990 with the frequency of infection. The Central Valley is stippled; it is bounded to the east by the Sierra Nevada, to the west by the Coast Ranges, and to the south by the Tehachapis. Shaded regions in the pie diagrams represent infection frequencies determined by characterizing isofemale lines established from inseminated females collected at the sites²³. To determine the incompatibility type of a line, three males were individually crossed to uninfected females and three females were individually crossed to infected males. The flies were 1–4 days old. Adults were discarded after two days and egg hatch was scored more than 25 h later. Crosses producing $>75\%$ egg hatch were scored as compatible and crosses producing $<25\%$ egg hatch as incompatible. Lines were scored as infected when crosses to infected males were compatible and crosses to uninfected females were incompatible. Crosses with uninfected lines produced the reverse. Ambiguous lines were retested. Confidence intervals (p_i, p_u) for each estimate were constructed by numerically determining the binomial parameter p , such that the probability of observing at least as many infected lines as observed with $p = p_i$ equals 0.025 and the probability of observing no more infected lines than observed with $p = p_u$ equals 0.025. The sample sizes, estimated frequencies and confidence intervals are: Arvin: 48, 0.90, (0.77, 0.97); Davis: 55, 0.04, (0.004, 0.13); Escalon: 48, 0.29, (0.17, 0.44); Gridley: 50, 0.06, (0.01, 0.17); Healdsburg: 52, 0.10, (0.03, 0.21); Ivanhoe: 63, 0.81, (0.69, 0.90); La Conchita: 41, 0.98, (0.87, 0.999); Piru: 65, 0.83, (0.72, 0.91); Riverside: 18, 0.94, (0.73, 0.999); Shandon: 25, 0.84, (0.64, 0.95); Solvang: 51, 0.90, (0.79, 0.97); Templeton: 52, 0.83, (0.69, 0.92); and Watsonville: 46, 0.93, (0.82, 0.99).

FIG. 2 Infection frequency at three sites over time. Frequencies were determined as for Fig. 1. Error bars are approximate standard errors calculated as $\sqrt{p(1-p)/N}$, where p is the estimated infection frequency and N is the number of isofemale lines scored. Confidence intervals, (p_l, p_u) , for each estimate were determined as in Fig. 1. For the two samples in which no infected lines were found, we computed a value of p_u such that the tail probability equals 0.05. The sampling dates, sample sizes, estimated frequencies and confidence intervals for each location are: Riverside/Highgrove: June 1985, 24, 0.96, (0.79, 0.999); June 1987, 27, 0.96, (0.81, 0.999); July 1989, 260, 0.94, (0.91, 0.97); and October 1990, 18, 0.94, (0.73, 0.999); Arvin: June 1987, 20, 0.0, $p_u=0.14$; November 1989, 65, 0.29, (0.19, 0.36); and October 1990, 48, 0.90, (0.77, 0.97); and Sanger/Ivanhoe: June 1985, 18, 0.11, (0.01, 0.35); June 1987, 7, 0.0, $p_u=0.35$; September 1989, 105, 0.27, (0.19, 0.42); and October 1990, 63, 0.81, (0.69, 0.90).



to approximately 34, 31, and 26 km per gen^{1/2}. A comparable figure emerges if we assume that the northernmost site with an infection frequency of 0.3 has moved ~175 km from Sanger to Escalon in one year. Using our estimate of ~15 generations per year in the Central Valley, this corresponds to a wave speed of approximately 12 km per gen and $\sigma \approx 45$ km per gen^{1/2}. All of these estimates of dispersal distances exceed by more than ten times direct estimates obtained from *Drosophila* release-recapture studies²¹⁻²⁴. Thus, the model of continuously distributed, demographically stable populations with gaussian dispersal distances that underlies equation (2) is probably inapplicable to these fly populations. Individual *Drosophila* can travel more than 10 km in a day²¹. Hence, long-distance migration, possibly aided by commercial fruit transport, may be more common than expected under the gaussian dispersal model. Also, population sizes plummet in the winter; and some populations may be re-established by immigrants from southern populations with high infection frequencies²⁴. In agreement with our results, patterns of genetic variation in many species indicate much higher dispersal rates than those directly observed²⁵.

To explain the simultaneous frequency increases at Arvin and Sanger/Ivanhoe, we conjectured that the infection entered the Central Valley not by crossing the Tehachapi range to the south,

but rather by invading from the west through the coast range³. This is supported by our samples west of the coast range, and at Shandon in a coastal valley that joins the Central Valley between Arvin and Ivanhoe. An alternative interpretation of our data is that the infection has not moved from southern to northern California, but rather has arisen independently and increased locally from frequencies that were too low to detect in our 1984 and 1987 samples. This alternative is not supported by mitochondrial DNA variation associated with incompatibility types²⁶. Infected strains from central and northern California share a restriction pattern with those from southern populations, whereas uninfected strains from northern populations where the R-type is rare or absent have a different restriction pattern (A.A.H., M.T. and S. W. McKenzie, unpublished data).

The rapid spread of the infection suggests its potential usefulness in manipulating insect populations. Any cytoplasmic factor will be maternally inherited along with the infection and will therefore spread rapidly in a population. Factors having a deleterious effect on fitness or increasing susceptibility to a toxin could thereby be introduced into a population^{27,28}. At least occasionally, infectious agents may cause reproductive isolation between species²⁹. As they can spread rapidly, they may be a potent force in evolution. □

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Homeobox gene expression correlated with the bifurcation process of limb cartilage development

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THE complex architecture of the limb cartilage pattern probably develops by the sequential segmentation and branching process of precartilaginous cell condensation under the control of positional signalling^{1,2} provided by the zone of polarizing activity (anteroposterior)^{3,4} and the apical ectodermal ridge (proximodistal)⁵. This signalling is monitored and interpreted in the mesenchymal cells and induces the position-specific response of subsets of genes. Homeobox genes may be responsible for the interpretation of signalling⁶⁻¹¹. A correlation between limb pattern and expression domains of the homeobox genes in the upstream region of *Hox/Chox-4* has been proposed¹⁰⁻¹². We have analysed the spatial expression pattern of the *Chox-1* genes during development of chick limb buds. In contrast to genes in *Hox/Chox-4* expressed coordinately along the anteroposterior axis^{10,11}, homeobox genes in *Chox-1* have unique and mutually exclusive expression domains along the proximodistal axis. We report here that the expression domains of the *Chox-1* genes are closely related to the segmental structure of cartilage along the proximodistal axis, whereas the expression domains of the *Chox-4* genes are related to the cartilage branching pattern.

Outgrowth of the chicken limb bud is visible from stage (st.) 16

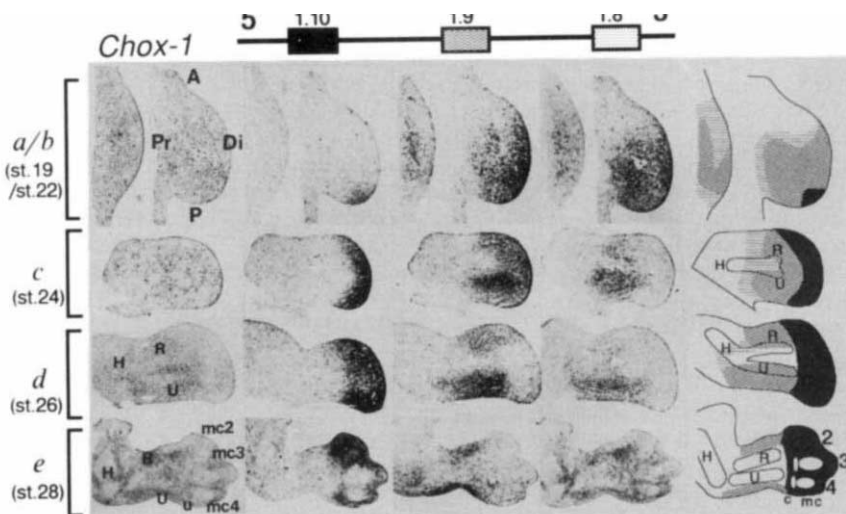
(ref. 13). The *Chox-1.10* transcripts were first detected in the mesenchymal cells in a small area of the posterior-distal region at st.22 (Fig. 1b). The *Chox-1.10* expression domain extended anteriorly and occupied the distal most region at st.24 (Fig. 1c). At st.28 (Fig. 1e), the precartilage pattern was distinct under a phase-contrast microscope and the *Chox-1* gene expression domains were distinguishable in relation to the position of each precartilaginous condensation. At this stage, the proximal boundary of the *Chox-1.10* domain was found between the autopodium and zeugopodium and the domain included both carpal and metacarpal elements. The *Chox-1.8* and *Chox-1.9* transcripts became observable in mesenchymal cells at st.19 (Fig. 1a). The *Chox-1.8* transcripts were found in nearly all mesenchymal cells. At st.22, the expression of neither gene was detected in the anterior proximal region of limb bud which is known to give rise to the shoulder³ and where the *XIHBx1* expression domain is located¹⁴. At st.24 (Fig. 1c), the *Chox-1.8* expression began to decrease and the transcripts in the region distal to the branching point of the Y-shaped aggregate were no longer detectable. By contrast, the *Chox-1.9* domain was found in a region where the *Chox-1.8* transcripts had disappeared. After st.26, the hybridization signals for *Chox-1.8* transcripts were greatly weakened. At st.26, as seen in the relationship between *Chox-1.8* and *Chox-1.9* domain at st.24, the *Chox-1.9* transcripts in the distal region disappeared where the *Chox-1.10* domain was located (Fig. 1d). At st.28, the proximal boundary of the *Chox-1.9* domain was in the region between zeugopodium and stylopodium and the distal boundary was in the region between autopodium and zeugopodium. In each case, the expression ceased in the cells integrated into precartilaginous condensation. Thus the expression boundary of *Chox-1* homeobox genes subdivided the limb bud along the proximodistal axis and the gene expression domains overlapped with the structurally segmented domains, zeugopodium and autopodium (Fig. 4b, d).

The *Chox-4.6* and *Chox-4.7* domains were observed in the posterior-distal region as almost overlapping domains. But the *Chox-4.6* domain was reproducibly wider than a *Chox-4.7*

FIG. 1 Expression domains of the *Chox-1* genes in the forelimb bud. The transcripts of the genes were visualized by non-radioactive *in situ* hybridization on neighbouring horizontal sections of embryonic limb at successive developmental stages. The far left panels show phase-contrasted views and the other panels show hybridization with the specific probes indicated. All photomicrographs are oriented with the anterior to the top and proximal to the left. The far right panel shows a schematic representation of each homeobox gene expression domain; each domain displayed is the same pattern as that indicated by the gene shown above. Stages of the embryo, indicated at far left, are according to ref. 13. The limb cartilage develops in a proximodistal direction originating from a single precartilaginous condensation at st.22. This aggregation elongates distally, then the distal end of the condensation branches to form the two elements of the radius and the ulna at st.24 (ref. 17). This Y-shaped structure continues its distal elongation until the complex is segregated at st.26. The elongation continues and ultimately produces the proximal carpal element.

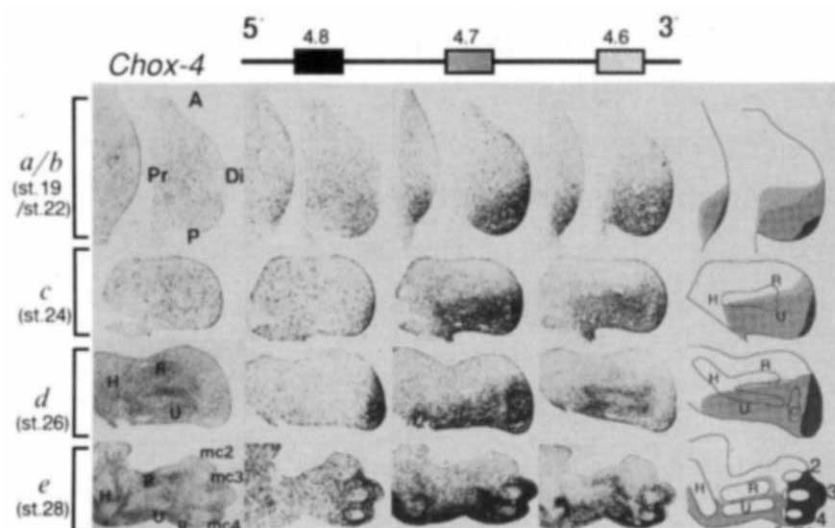
When the limb development reaches the stage of palm formation, the direction of pattern development switches to along the anteroposterior axis and produces the digits and distal carpus sequentially. At st.28, precartilaginous condensation of carpus and metacarpus are distinct². Abbreviations: H, humerus; R, radius; U, ulna; u, ulnare; mc, metacarpals; Pr, proximal; Di, distal; A, anterior; P, posterior.

METHODS. *In situ* hybridization was carried out as described¹⁸. The cryosections were hybridized with digoxigenin-labelled antisense RNA probes prepared from genomic DNA clones and monitored by enzyme-linked



immunoassay¹⁹. All material was oriented correctly during embedding and sectioned horizontally. Seven pieces of 5-μm serial sections were collected on seven slide glasses and seven serial sections were repeatedly distributed on the same seven slide glasses in the same order. Six of these were hybridized with six different probes and the seventh slide was used for histochemical analysis. Twenty-four limbs were analysed at each stage. More than 100 cryosections of limbs were examined after hybridization to each probe and all sections showed constant expression patterns.

FIG. 2 Expression domains of the *Chox-4* genes forelimb bud. Localization of the transcripts of three genes in the *Chox-4* was analysed by *in situ* hybridization as described in Fig. 1. Neighbouring sections of the embryonic limb bud were hybridized with probes for *Chox-4.6*, *-4.7* and *-4.8*. Structure of these genes in the *Chox-4* is shown at the top. Abbreviations are the same as in Fig. 1. The drawings in the far right panel are schematic representations of each homeobox gene expression domain of a given stage. The later domains were overlaid on the former. Sections used for the *Chox-4* probe hybridization were the serial sections originating from the limb buds used in Fig. 1. As in the case of the *Chox-1* gene, the expression was found in mesenchymal cells, and once cells were integrated into precartilaginous condensation the expression ceased. The expression pattern of *Chox-4* genes at earlier stages were essentially the same as already reported¹¹.



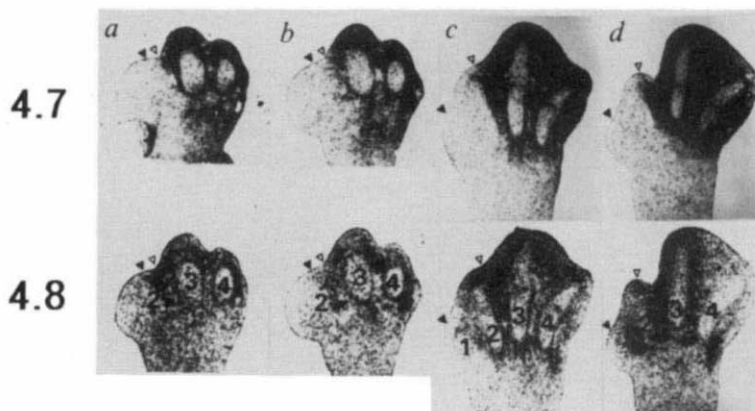
domain (Fig. 2a) and this tendency was even more obvious at st.22 (Fig. 2b). At st.24 (Fig. 2c), the anterior boundaries of the *Chox-4.6* and *Chox-4.7* domains were localized posteriorly to the humerus and across the branching point of the radius and the ulna. At st.28 (Fig. 2e), the anterior boundaries of the *Chox-4.6* and the *Chox-4.7* domains divided the radius and the ulna at the proximal region and then separated the metacarpus 2 and the metacarpus 3 at the distal region. *Chox-4.8* transcripts were found in a narrow and thin cell layer of the posterior distal region of limb bud at st.19 (Fig. 2a). At st.22, the *Chox-4.8* domain overlapped with the *Chox-1.10* domain but was narrower (Fig. 1b and 2b). At st.26 (Fig. 2d), the *Chox-4.8* domain expanded anteriorly and at the same time the *Chox-4.6* expression in this region was slightly repressed. The anterior boundary of the *Chox-4.8* domain was found just posterior to the metacarpus 2 and the proximal boundary was located between the carpus and the metacarpus at st.28 (Fig. 2e and Fig. 3). In addition, the anterior boundary of the *Chox-4.8* domain was found more anterior than the anterior boundary of the *Chox-4.6* and *-4.7* domains at st.28. This pattern was reproducibly observed in the neighbouring sections not only in the forelimb bud but also more distinctly in the hindlimb bud (Fig. 3). The digits are anticipated to be produced by a stereotyped bifurcation process which proceeds sequentially from posterior to anterior at the distal end¹. The expression of *Chox-4.8* expands from posterior distal to anterior, suggesting a correlation of the expression with this process. Thus the expression pattern of *Chox-4* genes coincided with the branching process of precar-

tilaginous condensation (Fig. 4c, e). The anterior boundary of murine *Hox-4.7* expression domain in limb bud was clearly separated by the anterior boundary of *Hox-4.6* domain¹¹, whereas separation of these two domains was not as obvious in chicken forelimb at any plane. This difference in mouse and chicken may be due to the difference in complexity of digit patterns between these animals.

As the resolution of this non-radioactive hybridization signal is at a single-cell level, coexistence of different homeobox gene transcripts in a single cell is easily examined. The hybridization signal was seen in nearly all mesenchymal cells in the domain, indicating that more than two homeobox genes of the same cluster are expressed in cells where the domains overlap. Thus the disappearance of the downstream gene transcripts in the region where two expression domains overlap at an earlier stage is due to the reduction in number of transcripts in the cells expressing two genes of the *Chox-1* cluster. There may be a regulatory interaction through protein products of the gene and the gene located just downstream in the cluster. This mutually exclusive expression pattern is not so distinct in the domains of *Chox-4* cluster genes, indicating a difference in the mechanism regulating target genes. Cells in the region where the expression domains of different cluster genes overlap, also expressed both cluster genes. But nothing was seen to suggest regulatory interaction between the two clusters.

The expression domains of *Chox-1* genes correspond to the segmental structure of cartilage along the proximodistal axis. Establishment of the expression domain was seen before the

FIG. 3 Detailed comparison of the anterior boundaries of the *Chox-4.7* and *-4.8* domain in autopodium level of limb buds at st.28. The hybridization pattern of *Chox-4.7* (4.7, upper row) and *-4.8* (4.8, lower row) to the sections from forelimb bud (a, b) and hindlimb bud (c, d) are shown. In each column the lower sections are just ventrally neighbouring to the upper sections. The sections in b and d are derived from regions 35- μ m more ventral than a and c in the same limb bud, respectively. At any level along dorsoventral axis, the anterior boundary of the *Chox-4.8* domain was anterior to the anterior domain of the *Chox-4.7* domain both in fore- and in hindlimb bud. This pattern was distinct at st.28 but was not observed at st.26. Sections are oriented with the anterior to the left and posterior to the right. Numbers in the figure indicate the number of metacarpals. ∇ , The anterior boundary of the *Chox-4.7* domain; $\blacktriangledown$, anterior boundary of the *Chox-4.8* domain.



appearance of the segmentation of precartilaginous condensation (Fig. 4b, d). Taken together, our observations suggest the involvement of the *Chox-1* genes in the segmentation process under the influence of the positional signalling along the proximodistal axis and regulatory interaction in the cluster. By contrast, the boundaries of the *Chox-4* gene expression domains are closely related to the branching process of precartilaginous

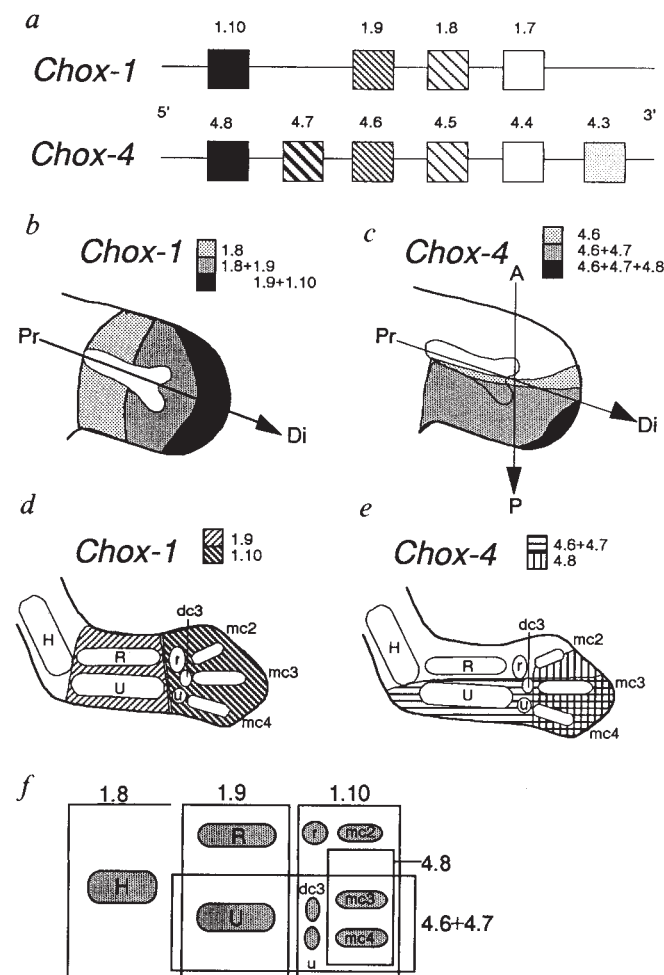


FIG. 4 Schematic representation of the cluster organization and the expression domains of the *Chox-1* and *Chox-4* genes in the forelimb. *a*, Organization of the chicken homeobox gene in upstream regions of *Chox-1* and *Chox-4*. The paralogous relationship of *Chox-1* genes with *Chox-4* genes is shown by the same patterns in boxes. This relationship is determined by comparison of the amino-acid sequence of the homeodomain (manuscript in preparation) with the sequence of the human homeobox gene in *HOX-1* cluster²⁰. *b* and *c*, Schematic representation of the dorsal view of the expression domains of *Chox-1* (*b*) and *Chox-4* genes (*c*) in the st.24 right forelimb bud at the plane around the central region. The precartilaginous condensation of the humerus-radius-ulna complex is drawn as a white Y-shape. The gene located nearer to the 5' region in the *Chox-1* has its expression domain in the more distal region of the limb bud. *d*, Correlation between chondrogenic pattern and expression domains of the *Chox-1* homeobox genes in the st.28 limb. The *Chox-1.9* domain contains the zeugopodium and the *Chox-1.10* domain includes autopodium. *e*, Correlation between chondrogenic pattern and expression domains of the genes in *Chox-4*. The ulna and its branching derivatives are included in the *Chox-4.6* and *-4.7* domain whereas the radius and its bifurcation derivatives are not. In contrast to the *Chox-1.10* domain, the *Chox-4.8* domain contains no carpal elements. *f*, This scheme illustrates a superimposed pattern of each expression domain through limb development in relation to the st.28 cartilage pattern. The boundary of each domain of the *Chox-1* genes corresponds to the segmentation process and those of the *Chox-4* genes correspond to the branching process. Abbreviations: Pr, proximal; Di, distal; A, anterior; P, posterior; H, humerus; U, ulna; R, radius; u, ulnare; r, radiale; dc, distal carpal; mc, metacarpals.

condensation (Fig. 4c, e). Transplantation of the zone of polarizing activity to the anterior margin of limb bud, which causes digital duplication, induced ectopic expression of the *Chox-4* cluster genes before the occurrence of visible pattern formation^{10,11}. This and our observation suggest the regulatory participation of the *Chox-4* genes on the branching process through interpretation of the anteroposterior positional signalling. As a difference in cell adhesion between proximal and distal mesenchymal cells was reported^{15,16}, it is possible that the *Chox-1* and *-4* gene products regulate the expression of the molecules which alter the cell adhesive properties that promote the bifurcation processes. The *Chox-1.10* and *Chox-4.8* domains initially appeared at the posterior-distal region and subsequently expand anteriorly. This suggests that together, the anteroposterior and proximodistal signalling initiate transcription and establishment of the domains. This is supported by the finding that ectopic expression of *Chox-1.10* is induced after retinoic acid treatment and *Chox-4.8* induction followed by the apical ectodermal ridge transplantation (manuscript in preparation). □

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Coalignment of vimentin intermediate filaments with microtubules depends on kinesin

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INTERMEDIATE filaments in most types of cultured cells coalign with microtubules. Depolymerization of microtubules results in collapse of vimentin and desmin intermediate filaments to the nucleus where they form a perinuclear cap (reviewed in ref. 1). Collapse can also be induced by microinjection of antibodies against intermediate filament or microtubule proteins^{2–7}. Thus, two filament systems interact with each other. But the molecules mediating this interaction are unknown. One of the candidates for this role is a microtubule motor kinesin⁸. Recent data showed that kinesin is involved in the plus end-directed movement of the membranous organelles along microtubules such as radial extension of lysosomes in macrophages⁹ and centrifugal movement of pigment in melanophores¹⁰. Here we report that injection of the

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anti-kinesin antibody into human fibroblasts results in the redistribution of intermediate filaments to a tight perinuclear aggregate but had no effect on the distribution of microtubules. Thus, kinesin is involved not only in organelle movement but also in interaction of the two major cytoskeletal systems, intermediate filaments and microtubules.

To study the role of kinesin in coalignment of intermediate filaments with microtubules, we used the microinjection of the affinity-purified rabbit antibody against the motor domain of the kinesin heavy chain (antibody HD) to knock out kinesin in human fibroblasts and analysed the distribution of intermediate filaments in the injected cells. This antibody inhibited kinesin-dependent gliding of microtubules and recognized a single band with a relative molecular mass of 120,000 (M_r , 120K) on immunoblots of bovine brain proteins¹⁰. Analysis of the specificity of interaction with fibroblast proteins shows that in AGO1523B cells the antibody also recognizes one component with a M_r 120K comigrating with the heavy chain of bovine brain kinesin (Fig. 1).

Microinjected cells were revealed with fluorescein-conjugated antibodies against rabbit IgG and intermediate filaments were stained in the same culture with a monoclonal antibody against vimentin and rhodamine-conjugated antibodies against mouse IgG. Anti-vimentin staining showed clearly that 4 h after HD injection intermediate filaments formed a tight perinuclear cap. Intermediate filaments in the injected cells were retracted from the leading lamellae as well as from the tail portions of the cells. Cells injected with preimmune IgG from the same rabbit were used as a control. None of them showed aggregation of intermediate filaments or their collapse (Fig. 2). Redistribution of intermediate filaments was evident 1 h after the injection of antibody HD when intermediate filaments started to accumulate in the central part of the cell, leaving most peripheral parts of the cytoplasm free of vimentin fluorescence (not shown).

Both control IgG and antibody HD were distributed uniformly in the injected cells (Fig. 2, inserts). Comparison of the fluorescence and phase-contrast images revealed that the injected antibodies spread into all cell processes and reached cell margins (not shown). Considering the data of Hollenbeck¹¹, who showed that kinesin concentration in fibroblasts is $0.16 \mu\text{M}$, one may calculate that by injecting about 5–10% (ref. 12) of the total cell

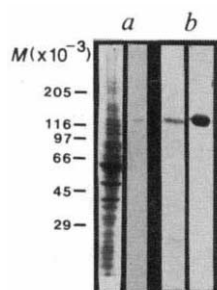


FIG. 1 Analysis of the antibody HD specificity by immunoblotting. The gels stained with Coomassie (a) and corresponding immunoblots (b). In each panel the right lane contains purified bovine brain kinesin and the left lane contains lysate of AGO1523B fibroblasts. The positions of M_r markers are indicated at the left.

METHODS. Antibody HD was raised against the head portion of the *Drosophila* kinesin heavy chain expressed in *Escherichia coli* (the construct was kindly provided by L. S. B. Goldstein and M. de Cuevas, Harvard University) and affinity-purified on the head portion of *Drosophila* kinesin. This antibody was characterized previously¹⁰. In some experiments additional chromatography on a Pharmacia FPLC system using a protein A-Superose column was done to concentrate the antibody and to remove traces of IgM that stained additional minor bands on immunoblots. AGO1523B human foreskin fibroblasts (National Institute of Aging Cell Culture Repository, Coriell Institute for Medical Research, Camden, New Jersey) were cultured in Dulbecco's modification of Eagle's medium supplemented with 10% fetal calf serum.

volume of $4\text{--}6 \text{ mg ml}^{-1}$ solution of IgG we introduced into the cytoplasm about 10-fold molar excess of the antibody over the cellular kinesin. Therefore, only a fraction of the antibody should be bound to its target, leaving most of the introduced proteins free to diffuse in the cytoplasm.

Intermediate filament distribution in fibroblasts is dependent on cytoplasmic microtubules. Therefore one may suggest that antibody HD induced collapse of intermediate filaments through its possible effect on cytoplasmic microtubule distribution. But immunofluorescent staining of the injected cells with a monoclonal antibody to tubulin and visual inspection of the stained cells shows that antibody HD had no effect on microtubule pattern (Fig. 3).

For quantitative analysis of intermediate filament aggregation in the injected cells we measured the ratio of the cytoplasm area occupied by intermediate filaments to the total cell area as originally suggested by Hollenbeck and Swanson for the analysis of lysosome extension⁹. The results of the measurements are presented in Fig. 4. For the control noninjected cells this ratio was 0.82 ± 0.09 (mean $\pm$ s.d.), confirming the visual impression that in such cells intermediate filaments occupied most of the

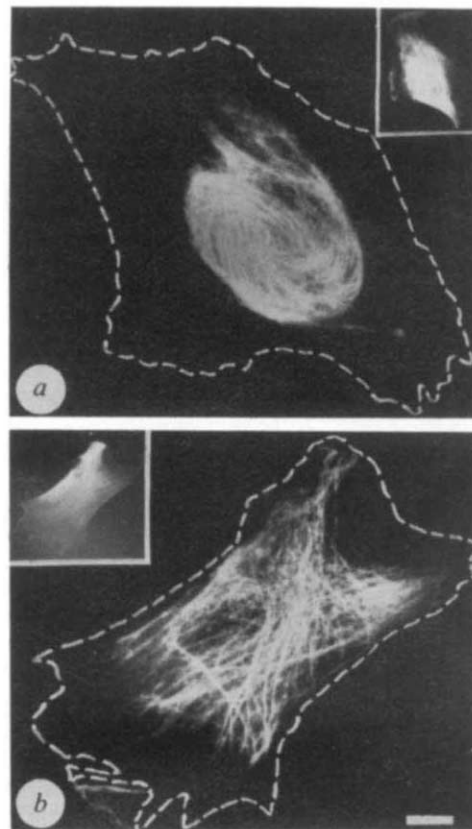


FIG. 2 Effect of antibody injection on the distribution of intermediate filaments in AGO1523B fibroblasts. a, A cell injected with antibody HD (4.2 mg ml^{-1}); b, A cell injected with preimmune IgG (6.3 mg ml^{-1}). Inserts, visualization of the injected antibodies in the same cells by staining with the fluorescent antibody against rabbit IgG. Cell margins are shown by white dashed lines.

METHODS. For injection, affinity-purified antibody HD was concentrated on a protein A-Superose column. Control IgG was isolated from the preimmune serum by chromatography on protein A-Superose. After injection cells were incubated for 4 h, fixed with 4% formaldehyde, permeabilized with 1% Triton X-100 in PBS and stained with a monoclonal anti-vimentin antibody NT30 (ref. 20) and a mixture of rhodamine-conjugated antibodies against mouse IgG and fluorescein-conjugated antibodies against rabbit IgG. Scale bar, $10 \mu\text{m}$.

cytoplasm. After the HD injection the ratio was greatly reduced to 0.25 ± 0.07 . For every injected cell tested, this ratio was lower than for any of the control cells. For the cells injected with preimmune IgG, the ratio was slightly less than for the control cells, although still higher than for the HD-injected cells (Fig. 4). This slight reduction of the area occupied by intermediate filaments after preimmune IgG injection can probably be explained by subtle cell rounding after the injection. The difference between all three groups of cells was highly significant ($>99.9\%$ according to Student's *t*-test).

These data show that kinesin plays a part in the organization of intermediate filaments in cultured fibroblasts and their coalignment with microtubules. Moreover, kinesin seems to be important not only for spreading of intermediate filaments into newly forming lamellar parts of migrating fibroblasts, but also for the permanent maintenance of their coalignment with microtubules. This is clear because anti-kinesin injection induced collapse of intermediate filaments not only from lamellipodia but also from the tails of the migrating cells. Additional evidence supporting this conclusion comes from our preliminary observation that anti-kinesin antibody injection into nonmigrating CV-1 cells located in the internal parts of the monolayer also produced collapse of vimentin intermediate filaments to the nucleus (F.K.G. and V.I.G. unpublished results).

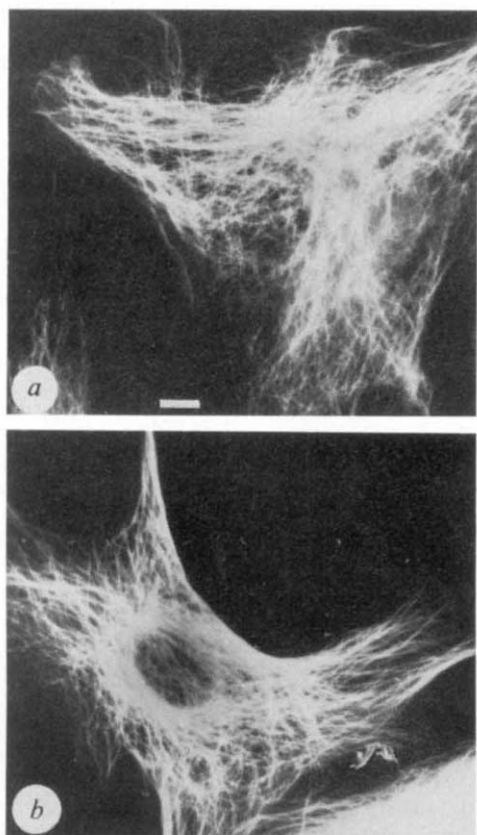


FIG. 3 Microtubules in the cell injected with antibody HD (a) and in the noninjected cell (b).

METHODS. Cells were injected with antibody HD (4.2 mg ml^{-1}). The culture was extracted 4 h after injection with 1% Triton X-100 in 50 mM Tris-HCl buffer pH 6.8 supplemented with 50 mM KCl, 0.5 mM MgCl_2 , 0.1 mM EDTA, 1 mM EGTA, 1 mM β -mercaptoethanol and 4% poly(ethylene) glycol 40,000 for 3 min, fixed with 1% glutaraldehyde and after reduction with NaBH_4 was stained with the monoclonal antibody DM1A (ref. 6) against α -tubulin (Sigma) and a rhodamine-conjugated antibody against mouse IgG. Scale bar, $10 \mu\text{m}$.

We suppose that coalignment of intermediate filaments with microtubules is the result of the equilibrium between two processes: kinesin-driven spreading of the intermediate filaments along microtubules and centripetal movement of intermediate filaments towards the cell centre. The latter process seems to be dependent on actin and ATP because, as was shown by Hollenbeck *et al.*¹³, the collapse of intermediate filaments after microtubule destruction was sensitive to cytochalasin and inhibitors of energy metabolism.

At present we can suggest at least two mechanisms of kinesin involvement in the positioning of intermediate filaments. The first mechanism is a direct interaction of kinesin (probably the tail portion of the molecule) with some protein of intermediate filaments. This protein is not vimentin itself because kinesin does not bind to purified vimentin filaments in sedimentation assay (A. S. Kashina, F.K.G. and V.I.G., unpublished results). Thus, kinesin may interact with some vimentin-associated protein. This idea is further supported by the results of Lin and Feramisco who showed that a monoclonal antibody against a 95K protein associated with intermediate filaments induced redistribution of intermediate filaments in cultured cells².

The second mechanism which now seems equally plausible is that the kinesin-dependent interaction of intermediate filaments with microtubules is mediated by the membrane-bound organelles. There is little doubt now that kinesin is involved in the plus end-directed movement of the membranous organelles along microtubules^{9,10,14,15}. Alternatively, intermediate filaments can interact with various lipids^{16,17} and at least some of the intracellular vesicles can bind to intermediate filaments^{18,19}. Thus vesicles powered by kinesin might move the intermediate filaments that are anchored to them. It is clear that whatever is the mechanism of kinesin interaction with intermediate filaments, this plus end-directed microtubule motor is involved in the interaction of two major cytoskeletal systems, intermediate filaments and microtubules. □

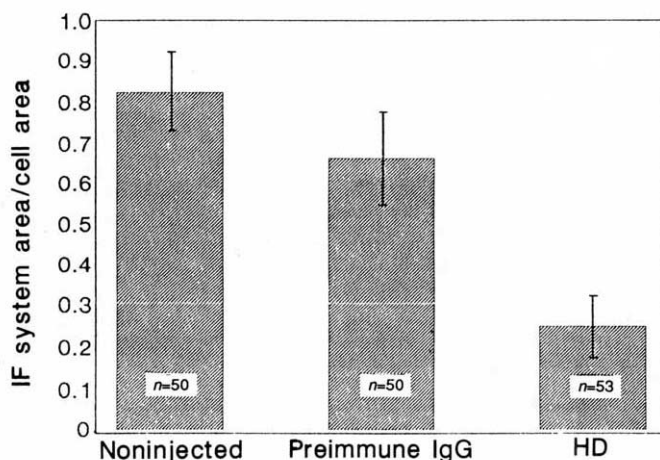


FIG. 4 Quantitative analysis of the effect of antibody HD injection on intermediate filament distribution. The histogram shows the ratio of the area occupied by the intermediate filaments to the total cell area (mean $\pm$ s.d.).

METHODS. Cells were processed for immunofluorescence as described in the legend to Fig. 2 and displayed on a TV-screen using a Zeiss Photomicroscope III equipped with a Hamamatsu 2400-08 SIT camera and a Hamamatsu Argus 100 image processor. Gain and offset settings on a camera control unit were adjusted in such a way that the ends of vimentin filaments were clearly seen on a screen in the rhodamine channel, the ends of filaments were connected and the area occupied by intermediate filaments was calculated using Argus 100 control program. The total cell area was calculated from fluorescent images in the fluorescein channel. The total cell area for the noninjected cells was determined after staining with fluorescein isothiocyanate ($1 \mu\text{g ml}^{-1}$). Validity of the procedure was checked by the measurement of the same cultures by two independent observers. These measurements always agreed to within 5% of the observed value.

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Cotranslocational insertion of apolipoprotein B into the inner leaflet of the endoplasmic reticulum

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APOLIPOPROTEIN (apo) B100 is required for the distribution of hepatic triglyceride to peripheral tissues as very-low-density lipoproteins. The translocation of apo B100 into the endoplasmic reticulum (ER) and its subsequent assembly into lipoprotein particles is of particular interest as the protein is both very large (relative molecular mass 512,000) and insoluble in water. It has been proposed that apo B translocation occurs in discrete stages and is completed post-translationally¹. Several sites of arrest of translocation were reported to be present in apo B15 (the N-terminal 15% of the protein). We have re-examined this question by *in vitro* translation coupled with translocation into microsomes, and find no evidence for transmembrane segments in truncated apo B proteins. Translocated apo B17 is strongly associated with the membrane of the ER, being only partially releasable with alkaline carbonate, and remaining bound to the microsomes following disruption with saponin. The efficient binding of short segments of apo B, despite the absence of transmembrane domains, suggests that apo B is cotranslationally inserted into the inner leaflet of the ER. This will obviate problems caused by the size and insolubility of apo B100, because the growing hydrophobic protein chains will never exist in a lipid-free form during translocation. From the inner leaflet, apo B in association with membrane-derived lipid can bud into the lumen of the ER to form nascent lipoprotein particles.

After biosynthesis apo B100 is either assembled into lipoprotein particles and secreted, or degraded intracellularly^{2,3}. Studies of cells in culture indicate that a proportion of the molecules are untranslocated and destined for degradation and that the remainder are translocated and quantitatively secreted². Studies of apo B15 translated in wheat-germ lysates have suggested that translocation pauses while translation continues, imposing transient transmembrane configurations¹; we cannot confirm these observations.

To obtain efficient translation of apo B in reticulocyte lysates, a partial 5' untranslated region (UTR) of mouse encephalomyocarditis virus (EMCV) (ref. 4) was fused to apo B complemen-

tary DNA. The EMCV-apo B transcripts encode MetAla before the apo B prepeptide sequence, but the extended signal still functions efficiently. Thus, apo B7 and B9 (Fig. 1a, b) increase in size after translation in the presence of microsomes owing to glycosylation⁵, and apo B9 is protected from trypsin digestion when translated with microsomes (Fig. 1a). When apo B7 is translocated in the presence of the competitive glycosylation inhibitor *N*-acetyl-AsnTyrThr-carboxyamide⁶, the principal product (unglycosylated apo B7) migrates faster than pre-apo B7, consistent with cleavage of the signal sequence (Fig. 1b). Cleavage (~90%) was confirmed by radiosequencing of processed apo B15 (results not shown).

In cultured cells apo B100 is carbonate-resistant^{7,8} immediately after biosynthesis, which indicates that integration into the ER membrane has taken place⁹. We therefore investigated whether the truncated apo B proteins bind to microsomes. Under conditions in which soluble secretory proteins are quantitatively released but a transmembrane protein will remain associated with the membrane, membrane binding was significant with proteins in the range apo B9 to B17, but less with apo B7 (Fig. 2a). Similarly, intermediate carbonate releasability has been found with apo E in Golgi membranes⁸; we have confirmed the membrane association of apo B17 following disruption with saponin (Fig. 2b).

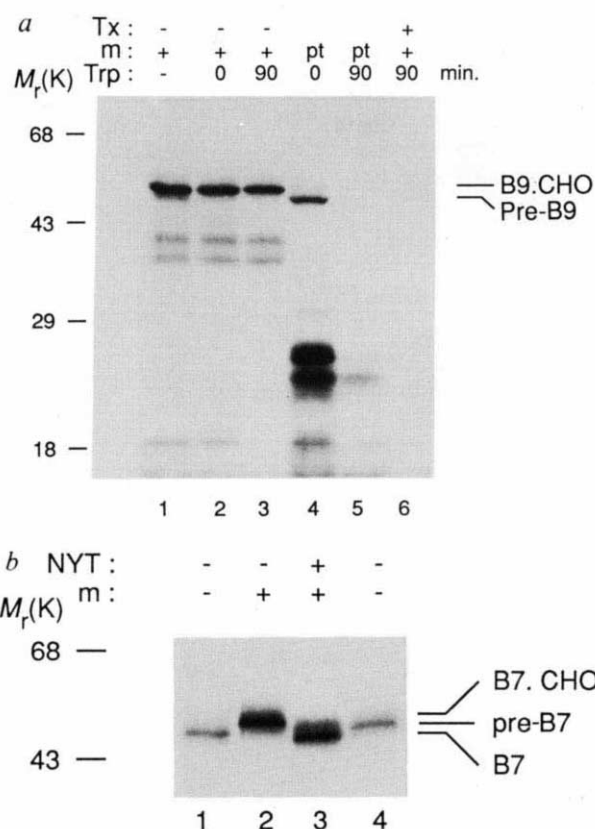
Association of apo B with the ER membrane could either involve amphipathic binding to the inner leaflet, or the presence of transmembrane regions. We observed that after translation in reticulocyte lysates for either 40 min (when protein synthesis is ongoing) or for 90 min at 30 °C, both apo B15 and apo B17 are protected from trypsin or proteinase K (Fig. 3a, b; and data not shown), thereby excluding the presence of transmembrane domains. To ensure reproducible inactivation of proteinase K, it was necessary to centrifuge the membranes out of the digestion mixture, but this protocol precludes the controls of post-translational addition of microsomes or total disruption with detergent to verify proteinase K susceptibility of apo B17. Instead it was shown that apo B17 becomes susceptible to proteinase K after the membranes are permeabilized with saponin (Fig. 3b). Although we did not find transmembrane domains in apo B, we confirmed that E₁ glycoprotein is a transmembrane protein (Fig. 3c)¹⁰.

Finally we determined whether generation of transmembrane domains resulted specifically from wheat-germ ribosomes. Reduced expression of some transmembrane domains in reticulocyte lysates relative to wheat-germ lysates has been reported^{11,12}, although the topology of proteins expressed in mammalian cells is better predicted by translation in reticulocyte lysates¹². But we find no evidence for transmembrane domains when apo B15 is translated in wheat-germ lysates and the microsomes are digested with trypsin (results not shown) or proteinase K (Fig. 3d). Using densitometry to quantitate full-length chains after digestion we find (1) 71% ± 13 s.d. (*n* = 9) recovery of apo B17 in reticulocyte lysates with trypsin and (2) 86% ± 31 s.d. recovery (*n* = 7) of apo B15 in wheat-germ lysates with proteinase K. In contrast, we find 0% (*n* = 4) recovery of apo B15 or B17 in saponin-disrupted microsomes after proteinase K digestion. Although we routinely included the membrane stabilizer tetracaine¹³ in digestion mixtures, we cannot detect transmembrane domains when tetracaine is absent but Ca²⁺ is present (results not shown)¹; in contrast, substantially reduced levels of putative intermediates have been observed with tetracaine present (S. L. Chuck and V. R. Lingappa, unpublished), which suggests that the data in ref. 1 may be a result of overdigestion.

We conclude that apo B is cotranslationally translocated into microsomes and binds to the inner leaflet of membrane. The binding of the amino terminus of apo B forms a focus for association of the remainder of the elongating protein with the membrane, circumventing the problems of size and hydrophobicity. It is probable that this amphipathic form of apo B

FIG. 1 SDS-PAGE showing expression of apo B7 and apo B9. **a**, Apo B9 was translated in the presence (tracks 1, 2, 3, 6) or absence (4, 5) of microsomes (m). To samples 4 and 5, microsomes were added post-translationally (pt). Samples were digested with trypsin (Trp) in the presence (track 6) or absence (tracks 2, 3, 4, 5) of detergent (Tx) or were undigested (track 1). Apo B9 is protected if cotranslationally imported into microsomes. Protected intermediates of synthesis (relative molecular masses (M_r) 38,000 and 40,000 (38K and 40K)) are apparent in some translations, but do not affect the interpretation of the experiment. The yield of apo B9 is stimulated by microsomes (tracks 1 and 4). When translation is carried out in the absence of microsomes (tracks 4, 5) peptidyl tRNA is apparent at ~25K. B9.CHO, glycosylated apo B9. **b**, Apo B7 was translated in the presence (tracks 2, 3) or absence (1, 4) of microsomes, with (track 3) or without (tracks 1, 2, 4) 100 μ M *N*-acetyl-AsnTyrThrcarboxamide (NYT)⁶. The peptide inhibited glycosylation of apo B7 to generate a protein species (B7) of faster migration than pre-apo B7, indicating cleavage of the MetAla signal sequence. B7.CHO, glycosylated apo B7.

METHODS. To generate the apo B9 transcript, construct EB9 was linearized with *Sall* and transcribed with T7 RNA polymerase (Boehringer). To generate the apo B7 transcript, construct EB9 was linearized at the *StuI* (1,199) site of the cDNA and transcribed. RNA was translated in unfractionated reticulocyte lysates (Amersham), with [³⁵S]methionine (Amersham) and, where indicated, in the presence of canine pancreatic microsomes (Promega). For protease digestion, translation mixtures were treated with 0.5 mg ml⁻¹ tosyl amino phenyl ethyl chloromethyl ketone (TPCK) trypsin (Cooper Bio-medical) for 90 min at 0 °C in 20 mM Tris-HCl, pH 7.5, and 2 mM tetracaine-HCl (Sigma)¹³. Where indicated, membranes were disrupted with 0.1% Triton X-100 during digestion. Digestions were terminated with 6 mg ml⁻¹ final concentration soybean trypsin inhibitor (Sigma). Proteins were resolved on 8→15% gradient SDS-polyacrylamide gels¹⁴. Subcloning: We used a plasmid (from M. Howell and T. Hunt) containing nucleotides 259 to 837 of the 5' UTR of EMCV with a novel *NcoI* site (underlined) created downstream of the first ATG (nucleotide 834) of the viral coding sequence: gattatATG GCCATGG. This encodes MetAla then the first Met and subsequent residues of any open reading frame cloned into the *NcoI* site. Apo B cDNA (refs 15, 16) was amplified by polymerase chain reaction (PCR) between nucleotides 128 and 400. A mismatched 5' oligonucleotide introduced an *NcoI* site at the first Met in the apo B signal sequence. The sequence¹⁷ of this product *NcoI*→PstI (394) showed a single nucleotide difference from the published sequence predicting Thr(40)→Ser. This was ligated to the *NcoI* site of the



modified EMCV 5' UTR and extended with apo B cDNA to encode: MetAla-pre-apo B9 (Ile(402)SerGluLysSerTer) (plasmid EB9); MetAla-pre-apo B11 (Thr(476)IleSerLysTer) (plasmid EB11); MetAla-pre-apo B15(Ala(691)-TyrArgTyrArgArg) (plasmid EB15); and MetAla-pre-apo B17 (Ile(782)LeuThr-LeuAlaGlySerMetGluSerProTer) (plasmid EB17).

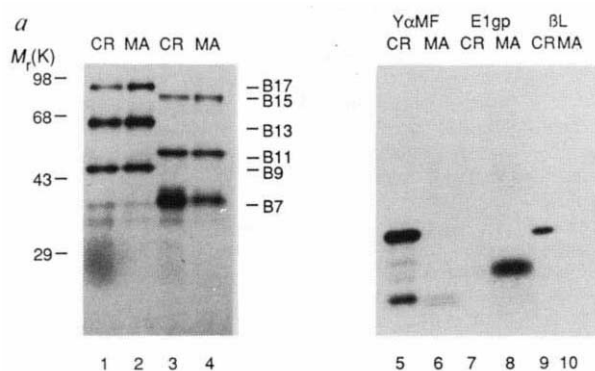
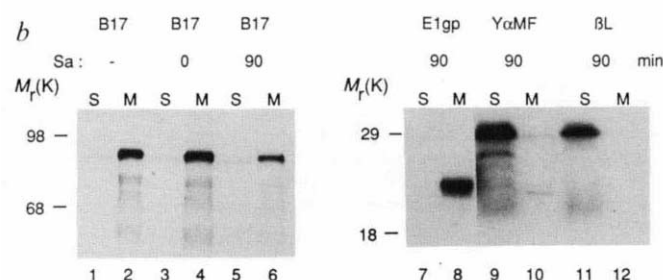


FIG. 2 Membrane of nascent apo B. **a**, Transcripts of apo B7 to B17 and of control proteins were translated in the presence of microsomes. Carbonate releasable (CR) and membrane associated (MA) proteins were resolved by SDS-PAGE. The tracks show: 1 and 2, mixed apo B9, B13, and B17; 3 and 4, mixed apo B7, B11 and B15; 5 and 6, yeast α -mating factor (YαMF); 7 and 8 E_1 glycoprotein (E1gp); 9 and 10, β -lactamase (β L). There is significant association of apo B9, 11, 13, 15 and 17 with the membranes, but apo B7 is mainly releasable with carbonate, (~80%). **b**, Apo B17 transcripts were translated in the presence of microsomes and were then either untreated (tracks 1, 2) or treated with saponin for 0 min (tracks 3, 4) or 90 min (tracks 5, 6). Transcripts of E_1 glycoprotein (tracks 7, 8), yeast α -mating factor (tracks 9, 10) and β -lactamase (tracks 11, 12) were translated in the presence of microsomes and treated with saponin for 90 min. Soluble (S) and membrane (M) fractions were separated and displayed by SDS-PAGE. Apo B17 is associated with the membrane fraction under conditions in which soluble proteins are released. Some apo B17 is present in the saponin-releasable fraction but migrates faster, and represents unprocessed pre-apo B17.

METHODS. To generate the apo B13 transcript construct EB17 was first



linearized at the *EcoRV* (1,952) site of the cDNA. RNA encoding β -lactamase and yeast α -mating factor was obtained from Promega. For carbonate extraction, carrier liver microsomes¹⁸ (~25 μ g) were added post-translationally to reticulocyte lysate translations and the membrane fraction was separated from the soluble fraction by centrifugation for 10 min at 30 p.s.i. in a Beckman airfuge. The membrane fraction was washed and resuspended in 200 μ l of 100 mM Na₂CO₃, pH 11.5, for 45 min at 0 °C. Membrane-associated proteins were separated from the carbonate releasable proteins by recentrifugation. For saponin extraction, carrier liver microsomes were added post-translationally and the mixture was then adjusted to 0.05% (final concentration) saponin (Sigma). The membrane fraction was separated from the soluble fraction by centrifugation. Proteins were resolved on 7→17% gradient SDS-polyacrylamide gels. Subcloning: cDNA encoding coronavirus E_1 glycoprotein¹⁹ was amplified by PCR with mismatched oligonucleotides to introduce an *NcoI* site at the ATG encoding first Met of the open reading frame and a *Sall* site immediately downstream of the termination codon. This was ligated to the *NcoI* site of the modified EMCV 5' UTR to encode MetAla-E1gp.

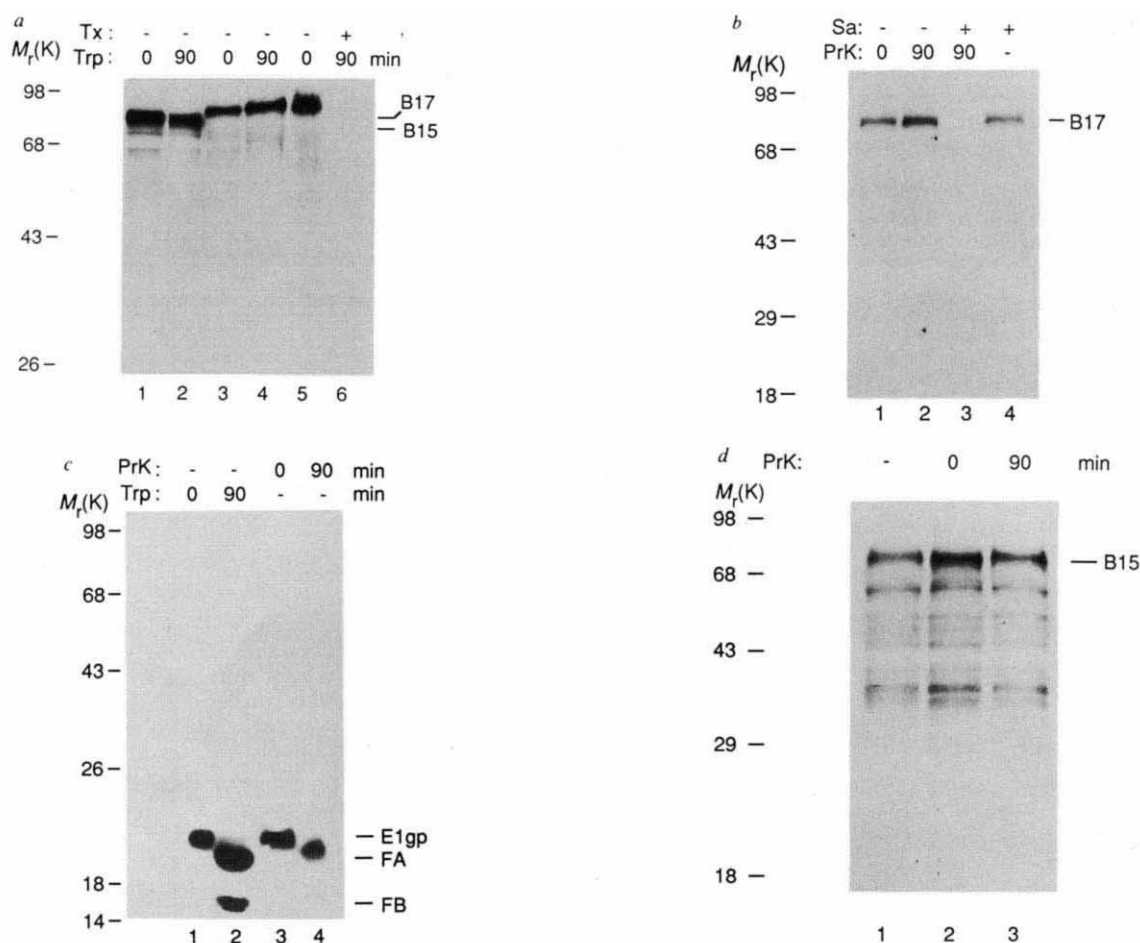


FIG. 3 Luminal disposition of apo B in microsomes. *a*, Apo B15 (tracks 1, 2) or apo B17 (tracks 3, 4, 5, 6) transcripts were translated in reticulocyte lysates in the presence of microsomal membranes for 40 min (tracks 3, 4) or 90 min (tracks 1, 2, 5, 6) and digested with trypsin for 0 min or 90 min. In tracks 6, 0.1% Triton X-100 (Tx) was included during the digestion. *b*, Apo B17 was translated in the presence of microsomes and then aliquots were left undigested (tracks 1 and 4) or were digested for 60 min with proteinase K (PrK) (tracks 2, 3) in the presence (3, 4) or absence (1, 2) of 0.05% saponin (Sa). Membranes were collected by ultracentrifugation. The results show that apo B17 is protected from proteinase K if cotranslationally translocated into microsomes, but not if the microsomes are disrupted with saponin during digestion. *c*, E₁ glycoprotein transcripts were translated in reticulocyte lysates in the presence of microsomal membranes and digested with trypsin (tracks 1, 2) or proteinase K (tracks 3, 4) for 0 min (tracks 1, 3) or 90 min (tracks 2, 4). E₁ glycoprotein (E1gp) was cleaved from a protein of apparent *M_r* 23.5K to a major product (FA) of 22K and a minor product (FB) of 19.5K (ref. 10), which migrates here at <18K. The 22K product is generated by cleavage of the cytoplasmic domain of E1gp. The smaller product is generated by cleavage of both the cytoplasmic and luminal domains¹⁰. *d*, Apo B15 transcripts were translated in wheat-germ lysate in the presence of microsomes for 60 min, and then the membranes were

collected by centrifugation before (track 1) or after digestion with proteinase K for 0 min (track 2) or 90 min (track 3). Some partial synthesis products are present in all tracks but no new bands are generated by protease digestion.

METHODS. Apo B15 capped transcripts were prepared by linearizing construct GB15 with *Sall* and transcribing with T7 polymerase in the presence of 0.5 mM GTP with 5 mM 7-methyl GpppG (Pharmacia), and fractionated on a Sephadex-G25 spin column (Pharmacia). Fractionated wheat germ lysate (Amersham) was reconstituted to 120 mM K⁺, with a 1 mM mixture of unlabelled amino acids excluding Met. Proteinase K digests were diluted with 2 volumes of 250 mg ml⁻¹ BSA in 0.15 M NaCl with 5 mM PMSF. Membranes were collected in an airfuge and washed 3 times with NaCl solution before dissolving in SDS loading buffer plus 5 mM PMSF for SDS-PAGE. In some instances membranes were disrupted with 0.05% saponin before protease digestion. Proteins were resolved on 8–15% or, for *c*, on 7–17% SDS-PAGE polyacrylamide gradient gels. Subcloning: The 50-nucleotide 5' UTR of human β -globin containing the natural *NcoI* site at the first ATG of the coding sequence²⁰ was synthesized as oligonucleotides with a 5' *SstI* site and a 3' *SstII* site to enable it to be cloned into the polylinker of plasmid pKS. Pre-apo B15 was constructed by ligation of *NcoI*→*HindIII* (2,279) fragments of cDNA to generate plasmid GB15.

subsequently buds into the lumen, carrying phospholipid and neutral lipids, to form the nascent lipoprotein particle⁹. □

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Telomerase primer specificity and chromosome healing

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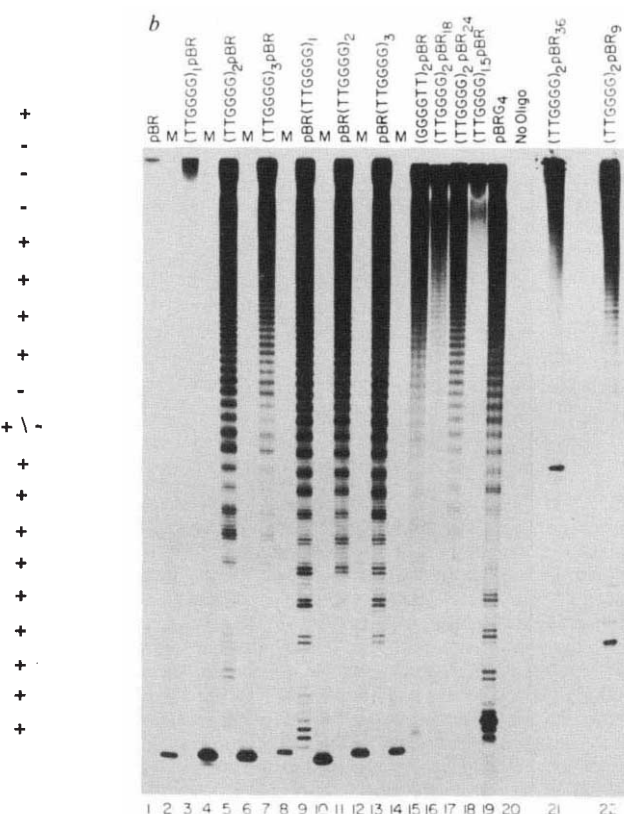
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CHROMOSOME healing by *de novo* telomere addition at non-telomeric sites has been well characterized in several organisms¹⁻⁹. The *Tetrahymena* telomerase ribonucleoprotein uses an internal RNA template to catalyse d(TTGGGG)_n telomere addition to the 3' end of telomeric sequence *in vitro* and *in vivo*^{10,11}. Studies of telomerase RNA indicated that hybridization of the RNA template region, 5'-CAACCCCAA-3', to the 3' end of single-stranded telomeric oligonucleotides might be important for primer recognition and utilization¹⁰. The apparent requirement of telomerase for pre-existing telomeric sequence has raised questions regarding its role in chromosome healing^{12,13}. We report here that *Tetrahymena* telomerase can specifically elongate single-stranded DNA oligonucleotides whose termini are not complementary to the RNA template sequence 5'-CAACCCCAA-3'. These data suggest that telomerase may be able to heal chromosomes directly *in vivo*.

<i>a</i>			
1	(TTGGGG) ₄	TTGGGGTTGGGGTTGGGGTTGGGG	+
2	pBRBam	AGCCACTATCGACTACGCGGATCC	-
3	α pBR	ATGATCGCGTAGTCGATAGTGGCT	-
4	pBR	AGCCACTATCGACTACGCGATCAT	-
5	pBRG ₄	AGCCACTATCGACTACGCACGGGG	+
6	pBR(TTGGGG) ₁	AGCCACTATCGACTACGCCTTGGGG	+
7	pBR(TTGGGG) ₂	AGCCACTATCGATTGGGGTTGGGG	+
8	pBR(TTGGGG) ₃	AGCCACTTTGGGGTTGGGGTTGGGG	+
9	(TTGGGG) ₁ pBR	TTGGGGTATCGACTACGCGATCAT	-
10	(TTGGGG) _{1.5} pBR	TTGGGGTTCTCAGCTACGCGATCAT	+/-
11	(TTGGGG) ₂ pBR ₉	TTGGGGTTGGGGCTACGATCAA	+
12	(TTGGGG) ₂ pBR	TTGGGGTTGGGGCTACGCGATCAT	+
13	(TTGGGG) ₂ pBR _A	TTGGGGTTGGGGCTACGCGATCAA	+
14	(GGGGTT) ₂ pBR	GGGGTTGGGGTTCTACGCGATCAT	+
15	(TTGGGG) ₃ pBR	TTGGGGTTGGGGTTGGGGGATCAT	+
16	(TTGGGG) ₂ pBR ₁₈	TTGGGGTTGGGGTATCGACTACGCGATCAT	+
17	(TTGGGG) ₂ pBR ₂₄	TTGGGGTTGGGGAGCCACTATCGACTACGCGATCAT	+
18	(TTGGGG) ₂ pBR ₃₆	TTGGGGTTGGGGCTACGCGATCATAGCCACTATCGACTACGCGATCAA	+
19	hu16	GAGGTGGGTGGGGCGAGCAGA	+

FIG. 1 Elongation of telomeric and non-telomeric oligonucleotides by telomerase. *a*, The name and sequence of each oligonucleotide is given; + indicates elongation, - indicates absence of detectable banding pattern under standard telomerase assay conditions and oligonucleotide concentrations less than 1 μM, as described previously²⁷. *b*, Telomerase reaction products resolved on a 10% polyacrylamide sequencing gel. Only selected oligonucleotides listed in *a* are shown. The ³²P-5' end-labelled primer oligonucleotides for some of the input primers are shown to the right of their respective telomerase elongation reactions (M; lanes 2, 4, 6, 8, 10, 12, 14). Test oligonucleotides that have been incubated with telomerase under standard reaction conditions are indicated above their respective elongation products (lanes 1, 3, 5, 7, 9, 11, 13, 15-19, 21, 22), including a control in

Telomeric oligonucleotides are elongated by telomerase, whereas non-telomeric oligonucleotides are not telomerase substrates¹⁴ (Figs 1 and 2). To test whether hybridization between the 3' end of primer oligonucleotides and the telomerase RNA template was an absolute requirement for elongation, a series of chimaeric oligonucleotides containing non-telomeric sequence and telomeric d(TTGGGG) repeats at either the 5' or 3' end were tested for elongation *in vitro* (Fig. 1*a*). One to three d(TTGGGG) repeats or d(GGGG), when placed at the 3' end of the non-telomeric pBR sequence, were sufficient for elongation. Strikingly, two or three repeats of d(TTGGGG) placed at the 5' end of oligonucleotides with non-telomeric 3' ends were also telomerase substrates. We tested chimaeric oligonucleotides containing two d(TTGGGG) repeats at the 5' end and from 6 to 36 bases of pBR sequence at the 3' end; all of these chimaeric oligonucleotides were elongated by telomerase (Fig. 1). The non-telomeric sequences at the 3' end of these oligonucleotides lacked complementarity to the telomerase RNA, although they did end in a single dT residue. But the same chimaeric oligonucleotides end in dA were elongated as efficiently. A 21 base, G-rich oligonucleotide corresponding to the junction of a healed, broken human chromosome 16 (ref. 9) was also a telomerase substrate (Fig. 1*a* and data not shown). The elongation of all primers was inhibited by preincubation of telomerase with RNase A, indicating that elongation of these chimaeric



which oligonucleotide was omitted from the reaction ('no oligo', lane 20). The banding pattern for oligonucleotide (TTGGGG)₂pBR₁₈ (lane 16) is weak on this gel, however in other experiments it is of comparable intensity to (TTGGGG)₂pBR₂₄.

METHODS. Standard telomerase assays were done as described previously²⁷. Partially purified¹⁴ or extensively purified telomerase was used (L.A.H. and C.W.G., manuscript in preparation). All oligonucleotides were synthesized by Operon Technologies, and were gel-purified. The full-length oligonucleotide was excised, eluted from the crushed acrylamide at 37 °C for 12-24 h in 500 μL sterile water, and purified over a Sepak C18 (Waters) or NAP-5 (Pharmacia) column as described by the supplier.

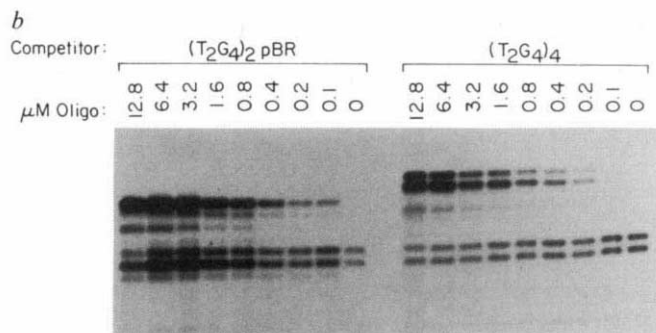
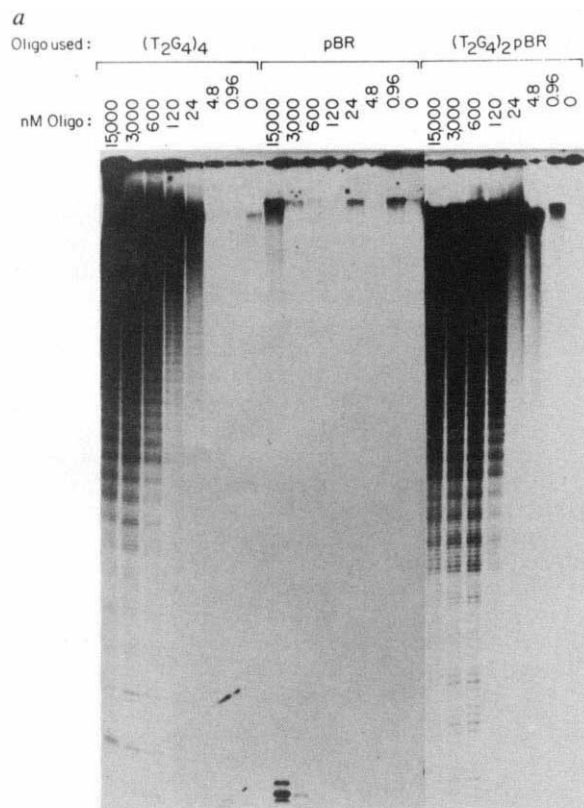


FIG. 2 Primer oligonucleotide titrations. *a*, Telomerase was reacted under standard conditions with primer oligonucleotide at a concentration ranging from 15,000 nM to 0.96 nM. The elongation products were resolved on an 8% sequencing gel. *b*, Telomerase reactions were carried out in the presence of 1 μ M 32 P-dTTP and no dGTP. Each reaction contained 150 nM d(TTGGGG)₃ and the concentration of competitor oligonucleotide is shown above the lane. The label incorporated into the end-labelled oligonucleotides was quantitated on the PhosphorImager. The ratio of signal in d(TTGGGG)₃ compared to d(GGGGT)₂pBR₁₂ from 12.8 μ M competitor to 0.1 μ M competitor was 0.3, 0.6, 0.94, 1.13, 1.19, 1.66, 3.74 and 5.5. The ratio of signal in d(TTGGGG)₃ compared with d(TTGGGG)₄ was 0.21, 0.27, 0.59, 0.81, 1.37, 1.85, 2.58 and 8.95. The signal for d(TTGGGG)₃ increased in the d(GGGGT)₂pBR₁₂ titration. This increase appears to be due to higher DNA concentrations, as a similar increase was found on addition of the pBR oligonucleotide and dT₁₈ (data not shown).

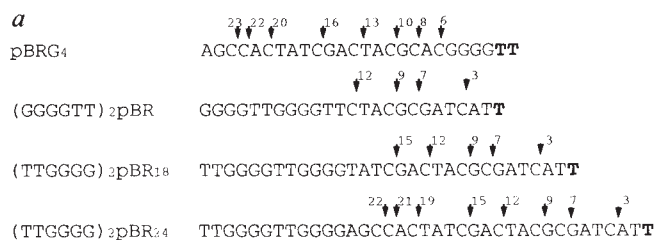
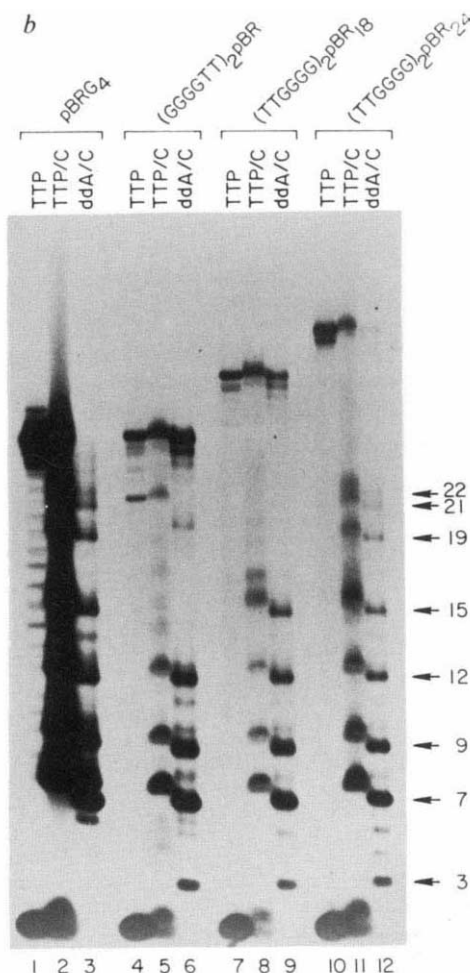


FIG. 3 Schematic diagram of telomerase end-labelled chimaeric oligonucleotides and the predicted cleavage products after Maxam-Gilbert cleavage at dC residues. *a*, The incorporated 32 P-TTP is in bold. The sites of cleavage are indicated with small arrows, and the numbers above indicate size of the predicted cleavage products. *b*, Maxam-Gilbert cleavage of telomerase end-labelled oligonucleotides. Only representative oligonucleotides are shown and are indicated above their respective lanes. The left lane in each set (TTP) shows oligonucleotide that has been incubated with partially purified telomerase and 2.4 μ M TTP. The second lane in each set shows the same telomerase end-labelled oligonucleotide after modification and cleavage at dC residues (TTP/C). Sizes of the digestion products (in nucleotides) are indicated to the right of the arrows. The right lane in each set shows the oligonucleotide 3' end-labelled with 32 P-dideoxyadenosine and cleaved at dC residues (ddA/C). The position of digestion products in lanes 2, 5, 8 and 11 is slightly higher than in lanes 3, 6, 9 and 12 because of excess salt and carrier DNA in the sample. For this reason, the three-nucleotide digestion products in lanes 2, 5, 8 and 11 are too smeared to see clearly; however, they are faintly visible on the original autoradiogram. Lanes 1 and 2 are overexposed in this exposure (6 days, -70°C). In this experiment, (TTGGGG)₂pBR was used in lane 6 instead of (GGGGT)₂pBR; these two oligonucleotides give identical digestion patterns after ddA labelling and cleavage at dC residues. Maxam-Gilbert cleavage was done essentially as described in ref. 28.



oligonucleotides, like that of telomeric sequences, requires telomerase RNA (data not shown).

To determine the affinity of telomerase for the chimaeric oligonucleotides, titration experiments were done. Chimaeric oligonucleotides with two d(TTGGGG) repeats at the 5' end were elongated at concentrations as low as 24 nM, as was the telomeric substrate d(TTGGGG)₄. The non-telomeric oligonucleotide was only a very weak substrate at oligonucleotide concentrations above 3 μ M (Fig. 2a).

To examine the initiation efficiency of chimaeric oligonucleotides compared with telomeric sequences, telomerase reactions were done in the presence of ³²P-dTTP alone. Under these conditions, two dTTP residues were added to oligonucleotides ending in d(TTGGGG), whereas chimaeric oligonucleotides with two d(TTGGGG) repeats at the 5' end were 3' end-labelled with a single dTTP. The relative efficiency of primer initiation was determined in competition experiments. The concentration of d(TTGGGG)₃ was held constant and increasing d(TTGGGG)₂pBR₁₂ or d(TTGGGG)₄ was added. The label incorporated into each oligonucleotide was quantitated using a PhosphorImager¹⁵. Both d(TTGGGG)₂pBR₁₂ and d(TTGGGG)₄ competed with d(TTGGGG)₃. The ratio of the signal in d(TTGGGG)₃ to the competitor oligonucleotide was similar for both titration series (Fig. 2b). Thus, the initiation of

the chimaeric oligonucleotides is of comparable efficiency to the d(TTGGGG)₄ telomeric substrate.

Three pieces of evidence showed that the elongation of chimaeric oligonucleotides with 5' d(TTGGGG) repeats was not due to removal of non-telomeric sequences. First, telomerase elongation products began above the position of the input marker oligonucleotide (Figs 1 and 2). Second, oligonucleotides incubated with telomerase in the presence of ³²P-dTTP migrated with full-length marker oligonucleotides on denaturing acrylamide gels (Fig. 3b). Finally, these end-labelled chimaeric oligonucleotides were subjected to Maxam-Gilbert cleavage at dC residues (Fig. 3b). Digestion products of the predicted sizes were obtained, providing further evidence that non-telomeric pBR sequences were retained during telomerase labelling (Fig. 3a).

Hybridization of telomerase RNA to the 3' end of primer oligonucleotides is not required for efficient initiation and elongation. When the 3' end of pBR oligonucleotides was held constant and d(TTGGGG) repeats were added at the 5' end, use by telomerase increased over 1,000-fold (Fig. 2a). Although elongation of non-telomeric oligonucleotides occurs only at high concentrations, the chimaeric oligonucleotides were elongated in the nM range. Thus, the presence of G-rich or telomeric sequence in an otherwise non-telomeric oligonucleotide greatly enhances the efficiency of initiation and elongation by telomerase.

We envision at least three models for the specific elongation of oligonucleotides with non-telomeric sequence at the 3' end (Fig. 4). In the first model, telomerase binds to the 5' end of a chimaeric oligonucleotide by RNA hybridization, and 'slides' to the 3' terminus to initiate d(TTGGGG) synthesis. Alternatively, the 3' non-telomeric sequences are 'looped out' to directly juxtapose the 3' end of the oligonucleotide with the RNA template. A third possibility is that telomerase recognizes telomeric DNA independent of telomerase RNA, for example, by DNA-protein recognition of G-rich sequences. This complex could then translocate (if necessary) to the 3' terminus of the oligonucleotide, to initiate d(TTGGGG)_n synthesis.

The telomeric oligonucleotide d(TTGGGG)₄ can form intramolecular G-G base-paired structures¹⁶⁻²⁰. Henderson *et al.* showed that oligonucleotides containing inosine substituted for guanosine, which do not form intramolecular base pairs, are elongated by telomerase²¹. In addition, oligonucleotides that form G-quartet structures inhibit *Oxytricha* telomerase²². Our observation that oligonucleotides with only d(TTGGGG) or d(GGGG) at the 3' end are elongated by telomerase supports the idea that intramolecular G-G base-paired structures are not required for telomerase activity.

It is interesting that new telomere addition on human chromosome 16 occurred 3' to a G-rich sequence⁹. The requirement for d(TTGGGG) repeats near but not at the site of telomere addition has also been found with yeast chromosome healing *in vivo*. Healing of linear plasmids and HO-induced chromosome breakage sites is stimulated by the presence of (TTGGGG)_n or G-rich sequences proximal to the healing site⁸ (K. Kramer and J. Haber, personal communication). HO endonuclease cleavage is associated with long single-stranded regions²³. Thus, substrates similar to the single-stranded oligonucleotides used here may occur *in vivo*. But, *de novo* telomere addition onto non-G-rich sequences is well documented in ciliates^{5,6,24}. In *Tetrahymena*, telomerase adds the first telomeric repeats onto A+T-rich sequences during chromosomal fragmentation (G.-L. Yu and E. Blackburn, personal communication). Although non-telomeric sequences are elongated only at high concentrations *in vitro*, the ability of telomerase to elongate non-G-rich sequences may be developmentally regulated, or tissue specific. For example, broken maize chromosomes are healed in the embryo but not the endosperm^{25,26}.

Telomerase can elongate oligonucleotides lacking telomeric sequences at their 3' end. These studies provide the first

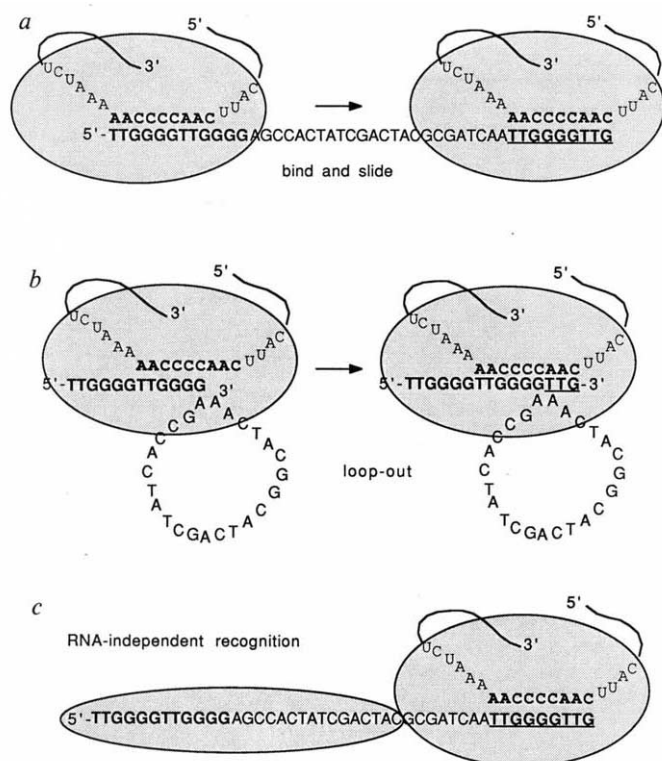


FIG. 4 Three models for telomerase elongation of non-telomeric termini. The telomerase RNA template region and complementary telomeric sequence are shown in bold. Newly synthesized d(TTGGGG) is shown in bold and underlined. *a*, Telomerase binds to the 5' telomeric or G-rich sequence through hybridization of telomerase RNA. After recognition, the complex would track to the 3' end of the oligonucleotide and initiate d(TTGGGG) synthesis. *b*, Telomerase RNA recognizes 5' G-rich sequences as in *a*. Non-telomeric sequences are looped out, retaining hybridization of the 5' end of the oligonucleotide, while bringing the 3' end close to the RNA template to allow d(TTGGGG) synthesis. *c*, Telomerase has an RNA-independent ability to recognize and bind G-rich structures which positions telomerase RNA at the 3' terminus of the oligonucleotide to initiate telomere addition. This model might also require tracking of telomerase to the 3' end of the oligonucleotide after binding.

biochemical evidence that telomerase is capable of directly healing chromosomes, and that hybridization of telomerase RNA to the 3' end of primer oligonucleotides is not required for elongation. The further characterization of telomerase will provide a more detailed understanding of the mechanism and regulation of this remarkable enzyme. □

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Recognition of a chromosome truncation site associated with α -thalassaemia by human telomerase

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TELOMERES define the ends of chromosomes; they consist of short tandemly repeated DNA sequences loosely conserved in eukaryotes ($G_{1-8}(T/A)_{1-4}$)¹. Telomerase is a ribonucleoprotein which, *in vitro*, recognizes a single-stranded G-rich telomere primer and adds multiple telomeric repeats to its 3' end by using a template in the RNA moiety^{2–6}. In conjunction with other components, telomerase may balance the loss of telomeric repeats due to DNA replication⁷. Another role of telomerase may be the *de novo* formation of telomeres. In eukaryotes like *Tetrahymena*, this process is an integral part of the formation of macronuclear chromosomes⁸. In other eukaryotes this process stabilizes broken chromosomes. A case of human α -thalassaemia is caused by a truncation of chromosome 16 that has been healed by the addition of telomeric repeats (TTAGGG)_n (ref. 9). Using an *in vitro* assay⁴, I show here that human telomerase correctly recognizes the chromosome 16 breakpoint sequence and adds (TTAGGG)_n repeats. The DNA sequence requirements are minimal and seem to define two modes of DNA recognition by telomerase.

The sequence of the chromosome 16 break point junction, ($\alpha\alpha$)^{TI}, implicated four positions where the chromosome could

have been truncated and telomeric repeats added (Fig. 1a)⁹. The primers –1/–25, –3/–25 and –4/–25, which span those four positions, all functioned as telomerase primers *in vitro* (Fig. 1b). The products of the –1/–25 and –3/–25 primed reactions were cloned and sequenced. The 3' ends of the primers were intact, and the DNA sequences added by telomerase generated the correct ($\alpha\alpha$)^{TI} junction sequence (Fig. 2a). These data suggest human telomerase can recognize the healing site and add the appropriate telomeric DNA.

Use of the primers by telomerase decreased when nucleotides were progressively removed from their 3' ends (Fig. 1b). This is consistent with the idea that base-pairing of the primer 3' end to the telomerase RNA template is important for recognition. Indeed, the substitution of an A for a G in the –3/–25 primer (primer –3/–25A), which would disrupt a base pair to the postulated telomerase template⁴ (Fig. 2a), decreased its use 2.5-fold (Figs 1c and 3a). The products of the –3/–25A reaction were shifted relative to the –3/–25 reaction (Fig. 3a), as if different nucleotides were initially added to the primer. Sequence analysis of the –3/–25A reaction showed the

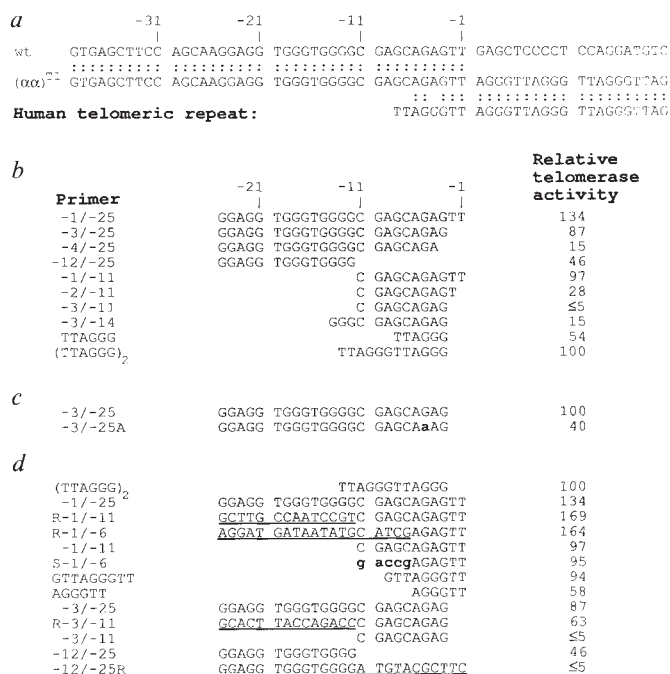


FIG. 1 DNA breakpoint sequence of the ($\alpha\alpha$)^{TI} chromosome 16 and relative telomerase activities of model oligonucleotides. **a**, DNA sequence of the ($\alpha\alpha$)^{TI} chromosome 16 compared to the wild-type (wt) chromosome⁸ and the human telomeric repeat. **b**, Telomerase primers derived from the ($\alpha\alpha$)^{TI} breakpoint. The primer names refer to their 3' (first number) and 5' (second number) ends in the breakpoint sequence (**a**). Relative telomerase activities are compared with the (TTAGGG)₂ primer (see Fig. 3). This primer is the most efficient of the primers (TTAGGG)_{1–4} (unpublished data). Examples of –1/–25, –3/–25, –12/–25, –1/–11 and –3/–11 primed reactions are shown in Fig. 3b. **c**, Telomerase primers used in Fig. 3a. The –3/–25A lower case boldface nucleotide is the base changed relative to the –3/–25 primer. The activities are relative to the –3/–25 primer. **d**, Telomerase primers with random DNA sequences used in Fig. 3b. The underlined nucleotides were randomly generated. The S-1/–6 lower case boldface nucleotides are the nucleotides rearranged relative to the –1/–11 primer. The activities are relative to the (TTAGGG)₂ primer.

METHODS. Dried polyacrylamide gels of telomerase reactions were exposed to Kodak storage phosphor screens and imaged with a Molecular Dynamics Phosphorimager. One-dimensional plots of each entire lane were transferred to Microsoft Excel, where the background was adjusted and the sum above background was determined. The lower limit of detection was ~5 units of relative telomerase activity. The values reflect the average of two to six experiments and are accurate to $\pm 15\%$.

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FIG. 2 Potential pairing of primers to the telomerase RNA template. *a*, The proposed base pairing of primers -1/-25 and -3/-25 to the putative telomerase RNA template⁴. The lower case boldface nucleotides are the nucleotides added to the primer 3' end as determined by sequencing clones of the telomerase products. The number of clones that had the observed sequence per number of analysed clones is indicated. *b*, Three possible pairings of the -3/-25A primer with the RNA template. The lower case nucleotide is the position of the mutation. The position column reflects the expected shift in gel mobility (in nucleotides) of the products relative to the -3/-25 primer. The per cent site usage column refers to the calculated usage of each position as compared with the -3/-25 primer. This was determined by quantitative image analysis assuming that each major product terminated in the same position in the telomeric repeat⁴. **METHODS.** Telomerase reaction products with greater than five added repeats were purified from a polyacrylamide gel. The products were amplified using the -12/-25 primer and the oligonucleotide CTGTCANNCTAACCTA by the polymerase chain reaction. Amplification conditions (100 μ l) were 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, 0.1 mg ml⁻¹ gelatin, 0.2 mM each dATP, dCTP, TTP and dGTP, 2.5 units *Taq* DNA Polymerase (Cetus), and 20 μ M each primer (94 °C 10 min, 1 cycle; 94 °C 2 min, 50 °C 2 min, 72 °C

telomeric DNA started at three different sites in the repeat sequence (Fig. 2*b*), consistent with different primer-template pairings directing the initial starting positions (Fig. 2*b*). The number of clones with each starting position agreed well with the relative telomerase utilization of each site (Fig. 2*b*). These data strongly support a 3' end base-pairing interaction where

<i>a</i>					
Primers	Primer-template pairing			No. of clones	
-1/-25	GGAGGTGGGTGGGGCGAGCAGAGTtagggttaggg...			10/19	
	3'-AUCCCAAUC-5'				
-3/-25	GGAGGTGGGTGGGGCGAGCAGAGTtagggttaggg...			6/6	
	3'-AUCCCAAUC-5'				
<i>b</i>					
Primers	Position	Primer-template pairing	Per cent site usage	No. of clones	
-3/-25A	-0	GGAGGTGGGTGGGGCGAGCAGAGTtagggttaggg...	~10	1/3	
		3'-AUCCCAAUC-5'			
-3/-25A	+1	GGAGGTGGGTGGGGCGAGCAAGAGTtagggttaggg...	~30	4/9	
		3'-AUCCCAAUC-5'			
-3/-25A	+2	GGAGGTGGGTGGGGCGAGCAAGAGTtagggttaggg...	~60	4/9	
		3'-AUCCCAAUC-5'			

2 min, 30 cycles; 72 °C 6 min, 1 cycle). The amplification products were gel purified, cloned into pGEM-3Z (Promega) and sequenced.

as few as two base pairs in the last four nucleotides are sufficient for telomerase recognition.

The region from -1 to -25 can be divided into two domains, a very G-rich domain which resembles telomeric DNA sequences (-12 to -25, 79% G), and a downstream region (-1 to -11, 36% G) that does not have the contiguous G residues typical of telomeric repeats. As internal telomeric DNA sequences can direct the addition of telomeric repeats to a downstream site in *Saccharomyces cerevisiae*¹⁰, the G-rich region was hypothesized as the site of telomerase recognition in the (α)^{TI} event⁹. The -1/-11 primer was efficiently used (Fig. 1*b*), demonstrating that the -12 to -25 G-rich region is not necessary for telomerase recognition. Nonetheless, addition of three Gs or the full G-rich region to the poorly used -3/-11 primer (primers -3/-14 and -3/-25; Fig. 1*b*) enhanced its priming efficiency.

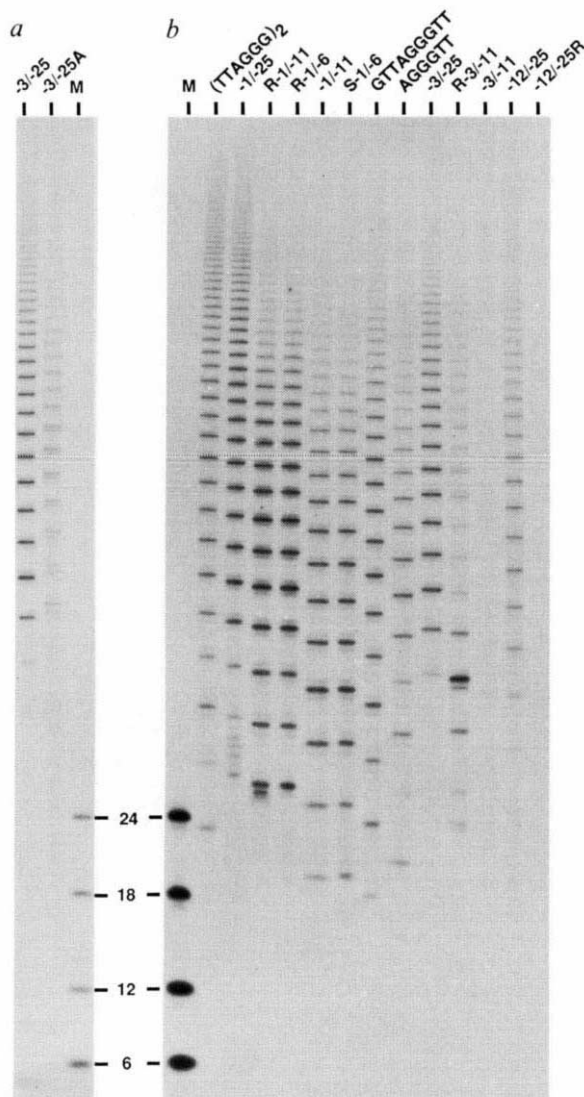


FIG. 3 Telomerase utilization of oligonucleotides from the (α)^{TI} breakpoint. *a*, The effect of a one-base change in the -3/-25 primer at the -5 position (-3/-25A; Fig. 1*c*). *b*, Comparison of primers with substitutions of regions from the (α)^{TI} breakpoint region with random sequences to their parent primers (Fig. 1*d*).

METHODS. Hela cell S100 extract was prepared as described⁴. A 45% ammonium sulphate precipitation was resuspended and bound to a DEAE Sepharose (Pharmacia) column. Telomerase was eluted with a 0.2–0.5 M KCl linear gradient and active telomerase fractions were passed over a S500 gel filtration (Pharmacia) column. Active fractions were stored at -70 °C. All experiments in this study were performed with the same telomerase preparation. The dGTP concentration and the reaction time used promoted the synthesis of long products⁴. The telomerase reaction was still in the linear phase at the 2 h point (not shown). The primer concentration used (10 μ M) was saturating for both the TTAGGG and (TTAGGG)₂ primers (not shown). Similar relative telomerase activities were obtained with a 2 μ M primer concentration (unpublished data). The relationships of these concentrations to the K_m 's of the thalassaemia primers are not known, thus the utilization measurements may not reflect the relative binding constants of these primers. Reactions were performed in 20 or 40 μ l final volume. Final reaction conditions were 50% telomerase extract (see below), 25 mM Tris (pH 8.2), 50 mM sodium acetate, 1 mM EGTA, 1 mM MgCl₂, 1 mM dATP, 1 mM TTP, 5 μ M α -³²P-dGTP (200 Ci mmol⁻¹) and 10 μ M primer. The telomerase extract contained 10% glycerol, 20 mM Hepes (pH 7.9), 50 mM KCl, 0.2 mM EDTA, 0.2 mM EGTA and 0.25 mM phenylmethylsulphonyl fluoride. The reactions were incubated at 30 °C for 2 h. The reactions were terminated and prepared as described previously⁴, except that the ethanol precipitation used 3 μ g carrier RNA. The pellet from the precipitation was suspended in 50 μ l 50 mM Tris (pH 8.0), 1 mM MgCl₂, 0.1 mM ZnCl₂ and 1 unit calf intestinal phosphatase and incubated at 37 °C for 30 min. Tris (20 μ l, 10 mM, pH 8.0), 1% SDS and 0.3 mg ml⁻¹ proteinase K were added and incubated at 50 °C for 30 min. The solution was extracted and ethanol-precipitated as described⁴. Samples were analysed on 8% polyacrylamide 8 M urea sequencing gels. Gel markers (M) were 5'-³²P-(TTAGGG)₁₋₄.

The ability of upstream G-rich sequences to stimulate primer use was investigated by replacing it with randomly generated sequences (primers R-1/-11, R-1/-6 and R-3/-11). The use of these primers compared favourably with primers -1/-25 and -3/-25 (Figs 1d and 3b). Thus, the stimulation seemed to be dependent on primer length rather than sequence. To investigate whether the -7 to -11 region fortuitously base-pairs with the telomerase RNA downstream of the template (this situation increases the activity of primers with other telomerases^{5,11}) these nucleotides were rearranged, with no apparent effect (S-1/-6 versus -1/-11; Figs 1d and 3b). Furthermore, replacing the -1 to -11 region of the -1/-25 primer with random sequence (primer -12/-25R) indicated that the G-rich region cannot efficiently activate downstream sites that pair poorly with the RNA template (Figs 1d and 3b).

The primer requirements of the ciliate telomerases suggested that 'G-quartets', a stable solution structure adopted by G-rich oligonucleotides^{12,13}, might be the recognized feature¹²⁻¹⁵. But recent experiments have shown that conditions that favour G-quartets in primers disfavour their use by telomerase¹⁶. My observations that G-poor primers such as R-3/-11 and R-1/-6 or short primers, like TTAGGG and AGGGTT, were efficiently used by human telomerase argues that primer recognition does not involve structures stabilized by G-G interactions. Rather, in support of an earlier model⁴, the oligonucleotides derived from the ($\alpha\alpha$)¹¹ breakpoint indicate two sites of primer interaction with telomerase (Fig. 4a) with the primer-template base-pairing interaction being the critical factor: only a primer

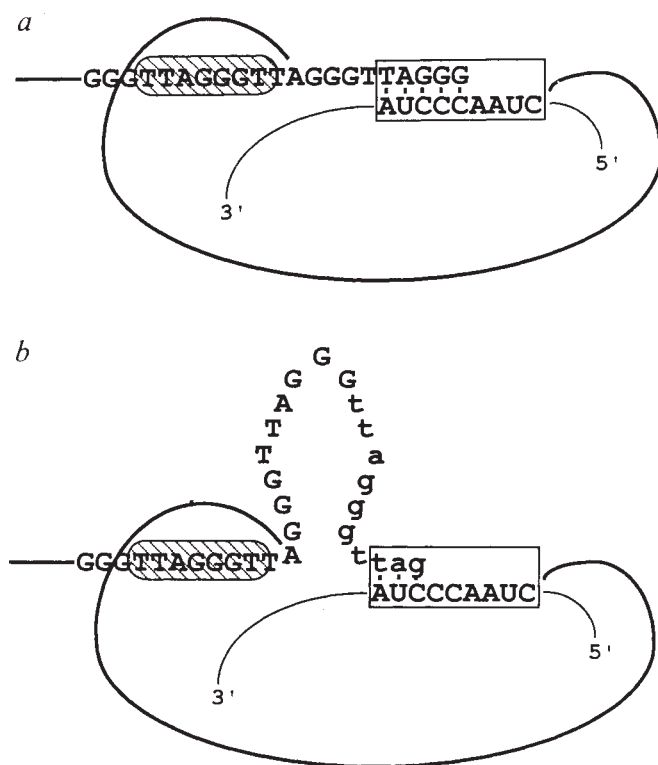


FIG. 4 Two-site model for primer recognition by telomerase. *a*, Recognition of primers by telomerase may occur at two sites on the enzyme. In the template site (rectangle) the telomerase RNA base pairs with the 3' end of the primer. The RNA template specifies the sequence of the new telomeric DNA synthesized in this site^{5,6}. The upstream site (hatched oval) interacts with regions of the primer 10-12 nucleotides upstream of the 3' end. *b*, Because multiple telomeric repeat addition requires repeated translocation events at the template site⁵ and the length of telomerase products depends on the length and sequence of the primer, the interaction with the upstream site may be maintained throughout many translocation-polymerization cycles. Thus, only the 3' end of the newly synthesized DNA (lower case letters) remains associated with the enzyme after translocation.

capable of stable base pairing to the template site was recognized efficiently (for example -1/-11). A second site, which interacts with the upstream region of primers longer than 10-12 nucleotides, affects the efficiency of primer use. But a strong interaction with this site was not sufficient to overcome a poor primer-template pairing (for example primer -12/-25R). Primers with a G-rich upstream region (-1/-25 and -3/-25) produced longer products than primers without a G-rich region (R-1/-11, R-1/-6, R-3/-11; Fig. 3b). This may imply (as previously suggested⁴) that addition of multiple repeats requires the newly synthesized DNA to translocate only at the template site, while DNA at the second site remains bound (Fig. 4b). In this way a G-rich primer might result in longer products by reducing the dissociation rate from the second site. The high use of the G-poor primers may be due to increased turnover.

The analysis of other chromosome healing events^{10,17-21} and the analogous process of telomere formation in ciliates⁸ have not revealed any DNA sequence requirements for telomere addition. Rather, chromosome fragmentation in *Tetrahymena* seems to be directed by 'chromosome breakage sites', which may initiate the formation of a terminal complex that mediates telomerase recognition²². Although my experiments with the ($\alpha\alpha$)¹¹ primers do not explain how telomerase recognizes a double-stranded end, they do suggest that 3' termini with only minimal complementarity (two to four nucleotides) to the telomerase RNA template can suffice for recognition. However, in ciliates, examination of telomere junctions where the parent micronuclear sequence is also known shows that about 50% could base-pair with the appropriate telomerase template (having at least two base pairs in the last three positions)²³⁻²⁹. Although other factors may mediate telomerase recognition in the context of ciliate macronuclear development, minimal base pairing with the template could be a means of recognition in the context of chromosome healing in ciliates and other organisms. Rare telomerase recognition events may be sufficient to stabilize a broken chromosome and allow its stable inheritance. Two other rare genetic disorders, the Miller-Dieker syndrome and the Wolf-Hirschhorn syndrome, also seem to involve terminal chromosome deletions^{30,31}. Terminal truncations are poorly characterized at present, and thus telomerase-based chromosome healing may be more common than believed. □

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Spreading the word

Abdus Salam

The developing world still needs more books and journals if its science is to flourish. There are many ways in which this literature could be provided.

KNOWLEDGE, that of science in particular, is transmitted through the spoken and written word. Ancient civilizations used parchments, tiles and paper for a somewhat reduced diffusion of their achievements among their contemporaries. The invention of printing in the fifteenth century accelerated the process until the Ulema stopped printing altogether in 1798, the date when Napoleon raided Egypt. Nowadays, not only is the number of books extremely high, but new technologies permit the collection, storage and retrieval of anything needed by whoever studies science or practices it as a profession. At least, this is true in the industrialized countries, where universities have comprehensive and specialized libraries, and where there are centres for scientific information processing, storage and retrieval.

When one turns to the countries of the developing world the picture becomes quite different, with the exception of a few prestigious research and training institutions such as the Indian Institutes for Technology. The situation is distressing — very few libraries, with very few books and journals. This is the rule, not the exception, either because foreign currency is scarce or because there is simply no money. One of the reasons why I left Pakistan in 1954 and made myself an exile was that not one library there had received any journals since the Second World War. Without literature, you cannot do any research.

No wonder that when visiting the International Centre for Theoretical Physics (ICTP) in Trieste, Italy, and seeing the library — 50,000 books, 1,000 subscriptions, and receiving 1,000 preprints each month — scientists from developing countries feel as though they are in paradise. For many of them, it is the first time that they have seen such a wealth of readily accessible information. Think of their frustration when they return home.

The issue at hand is really one of science transfer. The south needs science and technology in order to take off. Science is transferred through training in good places, working and discussing with experienced colleagues, and also through reading and studying good textbooks and consulting the relevant literature. This can be done only in the places where all these conditions are met. Unfortunately, most of these places are in the north,

certainly not in the poor countries of the south.

How to go about the provision of the printed word? We know the problem: there are many solutions, all of which are equally acceptable. (This, incidentally, is the difference between science and technology. Usually there is a unique answer in science to a proposition, whereas in technology there are hundreds of answers.) We must listen carefully to all the proposals.

Since the 1960s, when the developing countries won their independence, the number of scientists there has increased

ICTP DONATION PROGRAMME			
	Donations	Institutes	Countries
Journals	16,094	1,500	100
Books	1,791	1,500	100
Proceedings	3,640	1,500	100
Booklets	1,339	1,500	100

Data for October 1990 to May 1991.

considerably: there are now about 1,500,000, as opposed to the 2,000,000 in the advanced countries. The north has established libraries, some several centuries old. But the south has had to start from scratch, with the exception of the few countries possessing universities created by their colonizers.

I believe that it is possible to create viable libraries in developing countries, provided that there is strong solidarity among the whole scientific community. This solidarity in fact exists. In the 1970s, *Physics Today* published an appeal by me on behalf of the ICTP donation programme urging libraries to depart with their surplus of books and periodicals for the benefit of developing countries. Many did so and, for some time, my friend Luciano Fonda, a professor of theoretical physics at the University of Trieste and now scientific director of the Synchrotron High Radiation Laboratory, also in Trieste, coordinated the appeal, assessing the needs of developing countries and shipping books to libraries. Hassan Dalafi, my special advisor, is now in charge of this programme for both the ICTP and the Third World Academy of Sciences (TWAS). For the past four or five years, he has dispatched tens of thousands of items to 600 libraries in 93 developing countries (see table). He is also setting up a

database with UNESCO.

Let me now come to the cost of books and journals. One rarely finds a science book for less than 50 dollars, which represents 20 per cent of the GNP per capita in the developing countries, as opposed to 0.3 per cent in the rich countries. Everybody knows that there are 'pirate' editions of the Western scientific 'best-sellers' costing a fraction of the original. Therefore, it would be reasonable for the publishing house to abandon their copyright and to legalize the pirate practice. I made this suggestion at a meeting organized by TWAS in October 1989. My proposal met with an obstinate refusal, which is difficult to understand, especially bearing in mind that most publishers will not sell their books in these countries anyway. Some of them, however, have accepted to give the ICTP donation programme recent publications and proceedings. These are Springer-Verlag, World Scientific Publishing, North Holland, The Royal Society of London, the American Institute of Physics, the Japanese Physical Society, the Gothard Group, IUP Associated Press, Oxford University Press and Garland Publishing Company.

What about the copyright law for journals? Authors make no money by publishing their work in journals, unlike the authors of books. For journals published by professional societies, the institutions in which the authors work usually pay the society for publication privileges. The journal can then be sold cheaply. Commercial publishers, on the other hand, generally make no charges. But their journals are prohibitively expensive for developing countries. Again, would it not be reasonable for the publishers to abandon their copyright?

If publishers are reluctant to oblige, then what other ways are there of transferring literature? ICTP has another scheme for providing books and subscriptions. A committee selects and authorizes young scientists from developing countries to make purchases of up to 450 dollars each year for 4 years. There are 400 scientists involved in the scheme.

Second, UNESCO proposed in 1984 that a fund be established that pays royalties for the loan and photocopying of periodicals published by major international companies. About 500 periodicals were identified as being most needed. Subscriptions would be placed by local institutions, but funds would be administered by UNESCO. It was estimated that for a seven-year programme, the organization needed one million dollars per country. But the development agencies and other funding sources that were approached did not react positively to the proposal.

Alternatively, publishers could be induced to supply developing countries

with their publications at the fraction of their usual cost — perhaps one copy of each of the scientific journals to each of the countries.

But the simplest proposal in my opinion would be the one which the Chinese have adopted. In China, all scientific journals and books are reprinted at Hefei University and sold for a fraction of their price — usually one-third of a dollar. This be done because the country does not yet adhere to the copyright law. For the same reason, the university does not supply anyone else outside China. So far as journals are concerned, the university is supplying them to a set of people who would otherwise not be able to afford them.

What has been proposed so far is still limited in scope. At the Third General Conference of TWAS in November 1990, I suggested the establishment of central libraries in those developing countries where they do not yet exist. This proposal is based on my belief that every developing country should have at least one complete and up-to-date library. As a first step it was further suggested that the academy should start with a pilot project involving 5–10 countries, among them Egypt, Jordan, Morocco, Sudan, Syria, Tunisia and Turkey, with the support of international organizations. Professor Federico Mayor, director general of UNESCO, agreed that the organization would consider contributing an initial sum of 500,000 dollars. A feasibility study will begin soon. Members of the Italian Government have expressed their interest in this project, which fits the targets of the Italian programme for cooperation to development. If only governments of other industrialized countries of the north had this farsighted attitude.

To end, many authors of scientific books published by US, British or French companies are expatriates from developing countries, and they have achieved success and reputation in their new country. To them, I suggest they find a way in their contract with the publishers so that their work can also be printed cheaply in their country of origin.

New technology for the storage of information, CD-ROM for example, is useful and will become even more so in the future. But for many years still, books and journals will remain the vehicles of the written word in the everyday life of professional scientists. We must therefore solve the problem of how to provide the developing countries with these vehicles if science is to flourish there. □

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New journals review 1991

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- (1) the first number appeared between June 1989 and May 1990, with four issues available by May 1991 (although journals not covered in last year's review issue were also considered)*;
- (2) the journal is published at least three times a year;
- (3) the main language used is English;
- (4) where possible at least four issues should be made available for review, including the first and the most recent numbers.

Last year's journals review supplement covered publications appearing between June 1988 and May 1989, with four issues available by May 1990. The second cut-off date allows for enough issues of a journal to have been published for a reasonable sample to be available for judgement at the time of commissioning reviews.

Several journals known to satisfy the above criteria were not submitted for review, or arrived too late for inclusion. It proved difficult to find reviewers for other, doubtless worthy journals, while

some titles were considered to be of marginal interest to *Nature's* audience. Abstracts publications and newsletters are not reviewed. A list of titles eligible for review but not covered appears on page 481.

The brief given to the reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1991 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1991. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

*See *Nature* **347**, 581–599 (1990); **341**, 350–370 (1989); **335**, 459–478 (1988); **329**, 357–376 (1987); **323**, 359–379 (1986); **317**, 293–308 (1985); **311**, 309–330 (1984); **305**, 477–497 (1983); and **299**, 491–514 (1982).

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Carrying the Islamic torch

Ziauddin Sardar

Arabic Sciences and Philosophy. Editors Basim Musallem, Roshdi Rashed, Jean Jolivet, Muhsin Mahdi and George Saliba. Cambridge University Press. 2/yr. £48, \$75 (institutional); £28, \$39 (personal); £22 (members).

THE embryonic community of historians of Arabic and Islamic science and philosophy have been inadequately served by learned journals. Professionals in the field have tended to publish isolated articles either in established international journals of history of science such as

of the history of science and philosophy in general", paying "special attention to the history of scientific institutions, teaching systems and ideologies which made possible the emergence and development of the scientific and philosophical enterprise in medieval Islam".



Star-gazers: Arab astronomers (1513).

Isis, *History of Science* and *Archives Internationales d'History des Sciences* or national journals such as *The British Journal for the History of Science* and *Indian Journal of the History of Science*. Other outlets have included *Islamic Culture* published from Hyderabad, India, and the Pakistani journal *Hamdard Islamicus* — but these journals have too broad a canvas and have tended to be low on quality and high on self-congratulatory prose.

With the arrival, in the mid-1970s, of the *Journal for the History of Arabic Science (JHAS)*, published by the Institute for the History of Arabic Science of the University of Aleppo, the situation improved dramatically. Here at last was a journal that catered specifically for the professional historians of Islamic science. But *JHAS* lasted less than a decade: it died not so much from lack of contributions or demand but from political interference.

Some of the more noted historians involved in *JHAS* are now carrying the torch forward with *Arabic Sciences and Philosophy*. Launched following the establishment of the International Society for the History of Arabic Sciences and Philosophy in Paris, November 1989, the journal aims to 'crystallize' the 'tentative movement' behind the new disciplines. Covering the period from the eighth to the eighteenth centuries, it intends to "treat the history of Arabic science and the history of Arabic philosophy as part

Afro-Nature

Walter Gratzer

Discovery and Innovation. Editor T. T. Isoun. Academy Science Publishers, PO Box 14798, Nairobi, Kenya. 4/yr. Kenya Kshs 975, rest of Africa \$70, elsewhere \$90 (institutional); Kenya Kshs 700, rest of Africa \$38, elsewhere \$70 (personal).

In Yugoslavia, it seems, there are five biochemical societies, and they hold no joint meetings. Such unhappy Balkanization of the scientific estate is what the founders of this *Afro-Nature* hope presumably to nip in the bud. Their attempt to widen the African scientific and technological community does not go so far as to include South Africa, but the constituency, even so, is far from small. According to the editor, Professor Isoun, Nigeria in 1957 had one University (Ibadan) with 1,000 students. The population of Zaïre counted fewer than 10 graduates among its number. By 1989, when *Discovery and Innovation* was launched, Nigeria had 30 Universities with 150,000 students and Zaïre had accumulated 30,000 graduates. How many of these are scientists and how many lawyers is not revealed, but even so the momentum of scientific advance in black Africa must be considerable.

What need is there then, Professor

As such it casts a wide net seeking articles on Greek, Indian, Chinese, Latin, Byzantine, Syriac and Hebrew science and philosophy with a bearing on Arabic science and philosophy. The first two issues of *Arabic Sciences and Philosophy* carry articles in English and French, but the journal will also accept articles in Arabic; however, considering the readership of the journal, English is bound to become its dominant language.

Given the acute needs of its constituency, its wide but well-defined scope, as well as the professional reputations of those involved, *Arabic Science and Philosophy* is set to become the quality journal in the field. The only point against the journal, which incidentally many will also see as its basic strength, is that it appears to be the sole concern of scholars working in Western institutions: there is no one, either on the editorial board or on the advisory board, from outside Europe and North America. In this respect, too, *Arabic Sciences and Philosophy* needs to cast its net a little bit wider and bring in those isolated scholars working in the Middle East and elsewhere in the Muslim world. □

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Isoun asks, for a multidisciplinary journal, given the already abundant number of specialized journals in Africa, many of them now moribund? The answer must surely be that the practice of science now so transcends frontiers that the role of parochial journals is shrinking and research that is fit for print is migrating increasingly into supranational publications.

Discovery and Innovation is sponsored by the African Academy of Sciences, which is an offshoot of the Third World Academy of Sciences, based in Trieste. It is handsomely designed and produced, and contains a mixture of news, reviews and original papers, somewhat dominated by Kenya and Nigeria. Abstracts are in English and French. The topics are a mix of basic and applied. A review of biotechnology in the control of parasitic diseases that could have sprung from the pages of an American journal nestles beside dissertations on 'Production of non-fermented beverage from ripe bananas' and 'Effect of source of yeast on alcohol content and quality of pineapple wine'. The depredations of the cassave mealy bug *Phenacoccus manihoti* are juxtaposed with 'Boson critical self-mixing and the λ -transition in ^4He ' from Malawi and 'Approximation of fixed points of Lipshitz pseudo-contractive mappings in Banach spaces' from the University of Nigeria.

The journal will yield copious pickings for journalists, whether they are after

environmental catastrophe ('Lake Nyos gas explosion in Cameroon' from the University of Younde), straws in the economic and political wind ('New trends in nuclear power reactor technology' from the Atomic Energy Commissariat of Zaïre) or even culture ('On mathematical elements in the Tchokwe *Sona* drawing tradition' from Mozambique). How it will fare as an outlet for original research depends on how many African scientists choose to publish their best work there, rather than looking northwards. We should raise a glass of pineapple wine to its success. □

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East and West

Lucio Luzzatto

Biomedical Science. Editors Rem V. Petrov and Bernard T. Donovan. *Academy of Sciences of the USSR/Turpin Transactions/Pion.* 6/yr. Prices on application.

THIS is an unusual journal, because it identifies itself not by focusing on a specialized topic, but rather by being a Russian journal published in English. On page 1 of volume 1, G. I. Marchuk, president of the Academy of Sciences of the USSR and Sir George Porter, then president of the Royal Society of Great Britain, wished well to the new journal: their parallel statements give the impression that this is mainly a Soviet-British venture, although the editorial board is flanked by an international advisory board consisting of members from seven countries. The stated aim of the journal is "to present the best of Soviet science as applicable to health and medicine to a world audience". It seems that a two-tier reviewing system has been adopted, whereby manuscripts are first assessed by a Soviet panel, and then by international referees.

To what extent is the aim achieved? Judging from the most recent issue I have seen, papers are of a high standard, and they range widely from basic molecular biology, to classical physiology and biochemistry, to clinically oriented studies (such as two papers on lymphoid cells in patients with primary immune deficiency and in the synovial fluid of patients with rheumatoid arthritis). One subject conspicuous by its scarcity is genetics (a late side-effect of the Lysenko era?); the single exception is an excellent review on "problems in the control of genetic disorders", by A.M. Kuliev (formerly in charge of the Human Genetics Unit of the World

Health Organisation) and Bernadette Modell: this is also the only article that has a non-Soviet author.

Although *Biomedical Science* has not yet achieved the East-West dialogue that it intended perhaps to promote (there is no correspondence section), I would regard it as successful in terms of giving an image of ongoing biological research in the Soviet Union. Although I was unable to obtain absolute figures on circulation, I learnt that 30 per cent of subscribers are in the United States (as against 5-10 per cent in the Soviet Union). At the same time one wonders about two specific issues. First, for the journal to be effective one would wish to be sure that it is generally accessible to Soviet scientists both as authors and as readers: a statistically nonrepresentative sample of three that I recently asked did not know of its existence.

The other point is perhaps more important. There is no doubt that the West ought to take active steps to encourage a more prominent view of Soviet science on the international scene. There is also no doubt that, with a few exceptions, the vast scientific potential of the Soviet Union is at the moment severely under-expressed (some of the Eastern European countries have actually done better). But, to continue with current jargon, is the limiting factor for expression pre-transcriptional (the training of scientists), shortage of transcription factors (funding of research), or the presence of transacting repressors (bureaucracy), or is the main limitation at the translation level (the use of English)? I think the correct, if noncommittal answer, must be "all of the above".

Biomedical Science can at least remove the last hurdle, and to this extent it is a praiseworthy venture. If individual Western countries or the European Economic Communities now took the progressive step of earmarking funds for East-West collaborative projects, this journal could be a good forum for publishing results. In the long run, of course, one expects that the output of Soviet research should be reflected in 'regular' international journals, rather than in an 'ad hoc' publication. Those of us who have had an opportunity to know Soviet scientists in person have no doubt that this is what they desire and deserve. *Note added in proof:* So much has happened in the Eastern Republic in the past few weeks that this review sounds almost incongruous. But I have no doubt that the goal of more East-West dialogue in biology and medicine will now become even more important. □

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Risqué business

Roy Porter

Journal of the History of Sexuality. Editor John C. Fout. *University of Chicago Press.* 4/yr. US and Canada \$58, elsewhere \$61 (institutional); US \$29, elsewhere \$32 (personal).

SO LONG as sex itself remained a taboo subject, studies of the history of sex were mainly given over to veiled pornography (the history of the lash, and all that genre) or to dry-as-dust Germanic tomes on infibulation practices among obscure Amazonian tribes. The advent



Oedipus — myth or complex?

of sexual liberalization from the 1960s has at last brought the history of sexuality out of the closet. For the first time, for instance, we have a first-rate unexpurgated edition of Samuel Pepys's diary (R. Latham and W. Matthews (eds), *The Diary of Samuel Pepys*, 11 vols, Bell & Hyman, 1970-83), and the erotic preferences of James I or Catherine the Great can, at long last, be analysed without censorship or embarrassment.

The emergence of this new field of inquiry also owes much to the astonishing growth of social history over the last generation. Until recently, most professors focused on 'big history' (kings and battles). Now study of marriage and the family, of childhood and adolescence, of work and leisure, are all high on the agenda. Private lives as well as public events are given their due, and any study of private lives will necessarily give a central place to sex lives. Above all, the establishment, since the 1970s, of women's studies has inevitably drawn attention to matters sexual, because in patriarchal societies women were so often defined in exclusively sexual terms: madonna, whore, 'the sex'. Private and public fuse in the feminist study of

'sexual politics'.

Greater study of sex in history has created a far richer understanding of the subject. Under the influence of Freud, a rather simple-minded story was traditionally trotted out. History was a tale of libidinal repression. Natural instincts had been condemned by Christian 'thou-shalt-notism'; the sins of the flesh had been punished by the church and the courts. This long, frustrating chronicle of 'eros denied' had finally been changed by heroic sexual liberators, who had dared to challenge the taboo: Krafft-Ebing, Freud, Havelock Ellis, Kinsey and by brave oddballs such as Oscar Wilde and D. H. Lawrence.

Old-fashioned 'heroes and villains' readings of this kind are now under challenge, thanks in part to the welcome appearance of the new quarterly *Journal of the History of Sexuality*. For, as it is explained by its manifesto and affirmed by its contributors, sex is no longer interpreted in that rather hydraulic way, as a force of nature damned up, or allowed free flow, but as a repertoire of personal choices, integral to the fabric of culture and values at large, and inseparable from a politics of exchange and domination, class and race. How was it that mediaeval legends developed the figure of the prostitute saint? What does it mean to talk about 'homosexuality' in the eighteenth century (given that the term itself did not enter the language of psychopathology until the end of the nineteenth)? Articles posing such questions, and invoking the full variety of historical experience to challenge ingrained presuppositions about what is normal or perverse, make welcome appearance in the first numbers of the journal, alongside more traditional scholarship on, for example, the battles over the introduction of the condom into the United States.

Not the least virtue of this enterprising new venture is its genuinely interdisciplinary nature. An anthropological study of machismo in Morocco rubs shoulders with a literary analysis of hysteria in *Middlemarch*; a psychoanalytically inspired account of the novels of D. M. Thomas appears alongside a medical-historical discussion of syphilis in early modern Europe. If 'deviant' forms of sexuality are perhaps overrepresented in the early numbers, this simply reflects the exciting contemporary rediscovery of the gay and lesbian past, so long 'hidden from history'. To judge from its first four issues, the *Journal of the History of Sexuality* seems set fair to enhance our understanding of the historical interplay of biology and society. □

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Practical tips

P. E. Bryant

Psychological Science. Editor-in-chief W. K. Estes. *Cambridge University Press.* 6/yr. £72, \$110 (institutional); £35, \$55 (personal).

BROADLY speaking, psychologists are either academics or professional practitioners. As the subject has grown, the relative numbers of these two types of person have changed radically. The number of university teachers and of full-time research workers has undergone a steady but very modest increase over the years, but at the same time there has been a huge expansion in clinical, educational and industrial psychology.

One effect of this difference in the growth of these two branches has been a radical shift in the balance of power in various national psychology associations, and also a marked change in the atmosphere of their annual meetings. There was a time when the yearly meetings of the American Psychological Association and of the British Psychological Society were dominated by university professors, and the people who came to these meetings seemed interested only in the latest academic theory, however abstruse this was. Now a large proportion of the attendants wants to hear only discussions about practical issues, even though these are often not soundly based on research. Talks on academic research are less popular than they used to be and as a result research workers have begun to feel out of place at these meetings.

The consequent strain among academic psychologists in America recently led a number of them to form a new society — the American Psychological Society — which is a national organization exclusively for psychologists whose main interest is in scientific research. Many American academic psychologists have joined this organization; some of them in doing so left the American Psychological Association at the same time. Others, I gather, decided to be members of both organizations. The society has been holding its own annual conferences — dedicated to scientific research — and it has decided to mark its existence with a new journal, *Psychological Science*.

The journal has, as one might expect with a background like this, a certain parochial air, although one should bear in mind that this particular parish is a large and important one. Each issue starts with a two-page article entitled 'Psychology in Washington', which deals with the relations between current political events and psychological research,

and the topics discussed in these opening articles are often of little interest for non-American psychologists. The journal also gives news about the American Psychological Society ('First APS convention a smashing success'), but apart from this the articles do have a general interest.

The editor's aim is to bring academic psychologists together, and he sets out to achieve this by publishing papers that cover a wide range of psychological topics. The journal includes three main kinds of paper. Two of these are easy to describe and are generally very successful. One is the 'general article', which is usually written by a leading psychologist and which covers a particular topic in a fairly broad way. The second is the 'research report', which is a paper about a single empirical study written in a way that will be understood by any psychologist. The first issues include general articles by George Miller on language and by Sandra Scarr on child care, both of which are extremely well written and very persuasive. The research papers are without exception lucid and interesting. It looks as though the journal will keep psychologists informed about discoveries in other branches of the subject which might not otherwise come their way.

I am less sure about the third kind of article. In each issue there is a 'feature review', which is in effect two or more reviews written by different people on the same book. In theory, this seems like a good idea: different reviewers ought to produce different — perhaps even controversially different — views of the same product. But this does not seem to have happened, at any rate in the first few issues. The reviewers tend to speak with one voice, and a pretty uncritical voice at that. I see little point in these choruses of praise for books by illustrious colleagues, especially when these are books that I have read and which I know to have quite serious limitations. If the editors cannot find daring reviewers, perhaps they should choose books by authors whom their reviewers would dare to criticize.

In every other way the journal is a successful one. It satisfies a need in its own country and it should interest psychologists in the rest of the world as well. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1992. This information is not complete in all cases. Readers interested in a particular journal should check prices before subscribing.

Connecting threads

Peter Davies

Cerebral Cortex. Editors P. S. Goldman-Rakic and P. Rakic. *Oxford University Press*. 6/yr. US \$190, elsewhere \$220 (institutional); US \$95, elsewhere \$125 (personal).

Hippocampus. Editors David G. Amaral and Menno P. Witter. *Churchill Livingstone*. 4/yr. US \$130, elsewhere \$154 (institutional); US \$75, elsewhere \$99 (personal).

Dementia. Editor-in-chief V. Chan-Palay. Karger. 6/yr. UK £277, US \$425, elsewhere SwFr. 637 (institutional); UK £62, US \$94, elsewhere SwFr. 141 (personal).

THESE three new journals cover areas of interest to many neuroscientists; *Nature* provided my first chance to see them, as none has yet arrived in my department's library. *Cerebral Cortex* is clearly a superior product, and, with its reasonable personal subscription rate, is one of a short list of journals to which I shall subscribe. All of the papers in the first issue are substantial and thoughtful, and neuroanatomical, neurophysiological and computational studies are all represented. The quality of the illustrations, including colour plates, is splendid. If the editors can maintain the high standard of both the manuscripts and production, then this journal will become an important forum for publication of research papers.

I was less convinced of the need for *Hippocampus*, because this brain region is intimately associated with the cerebral cortex. Almost everyone working on the brain looks at this pretty region from time to time, and I suspect that if the journal was in the library, I would browse through it when time allowed. Because neuroscience journals do in general tend to serve particular subdivisions (for example, *Journal of Neurochemistry*, *Journal of Neurophysiology*), perhaps there is a need for a journal that considers structure from several different viewpoints. The quality of the papers in the first two issues is generally high, and again, the quality of the figure reproduction is good.

Because research of dementia is a particular interest of mine, I was especially interested to see the first issues of *Dementia*. I was pleasantly surprised to find that the journal lived up to the aims of its editor: to provide a forum for a broad range of papers devoted to this increasingly popular area of research. Contributions to the first four issues range from neurochemistry to case re-

ports, of higher quality than I had expected. Most of the papers are from European groups, and the journal will help US researchers keep up on what is going on across the Atlantic. (The editor notes that research findings on dementia are scattered among many other, less specialized journals. This is certainly true: for example, the first issues of *Cerebral Cortex* and *Hippocampus* contain articles on the related subject of Alzheimer's disease.) The price of subscribing to *Dementia* is relatively high, but probably worthwhile for any institution claiming to have a serious interest in the field. □

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Nervous beginnings

Eric A. Barnard

Molecular and Cellular Neurosciences. Editor-in-chief Michael Cann. *Academic*. 6/yr. £143.50, \$208 (institutional); £74.50, \$108 (personal).

YET another new journal in molecular neurobiology? That may be the initial reaction of many neuroscientists. But when I reviewed the new *Journal of Molecular Neuroscience* in *Nature* last

that carries consistently good quality papers and does not appear arbitrary in its selection for the benefit of a coterie. *Molecular and Cellular Neurosciences* will be, in my opinion, a welcome new entrant if it can help to overcome such obstacles. It aims to cover all areas of neurosciences at the molecular, cellular and tissue levels. Hence it should cater for a wide readership and authorship.

The new title is extremely similar to *Cellular and Molecular Neurobiology*, which is now in its eleventh year and has essentially the same prospectus. The mix of topics covered in the two is, so far, very similar. It will be interesting to see if the new offering can differentiate itself from the established one.

The journal started in 1990 with three issues (between August and December) and has now moved on to publishing six per year. It will need to become monthly before it can overcome the difficulties outlined above. Many of the papers are typical of such an early phase, not being substantial contributions, but several are of high quality and important in their field. The advantages that this journal has over its competitors include a striking, bright and interesting illustration on the cover of each issue (making it stand out on the library current-periodicals shelf), a large format, excellent printing and pictorial production, and a rapid publication schedule. In the most recent issues this averages 2 months or less from the received date to publication. If the journal can manage consistently to appear in libraries on its publication

date, then it should attract good papers, because this is not a journal merely for brief communications or for 'camera-ready' exhibitionism.

The journal has taken a wide field as its target. There is nothing intrinsically wrong with this – a few general journals are of value in every field, and their circulation certainly ought to increase. But each new addition will find it increasingly more difficult to establish a flow of quality contributions than will a specialist journal, where immediate recognition and interest go in its favour. The new journal has therefore taken

on a difficult task, but deserves to be given the chance to show whether it can excel in it. □

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Dendrites of rat pyramidal cells. (From the *The Enchanted Loom* edited by P. Corsi, Oxford University Press.)

year I noted that this is now probably the most rapidly growing field in biological science and that the market for publishing in it is not saturated. Those working in molecular neurobiology, and in some areas of cellular neurobiology, can still find difficulty in getting their papers published quickly in a journal

Brutal times

Michael Hanley

Seminars in the Neurosciences. Guest editors. Saunders Scientific. 6/yr. US \$120, elsewhere \$145 (institutional); US \$64, elsewhere \$85 (personal).

Comments on Developmental Neurobiology. Editor-in-chief Alain Prochiantz. Gordon and Breach. Variable number per year. US \$250, elsewhere Dfl. 550 (institutional); US \$131, UK £84, rest of Europe ECU 139 (personal).

Neuroreport. Editor-in-chief D. Ottoson. Rapid Communications of Oxford. 12/yr. US \$685, elsewhere £380 (institutional); US \$295, elsewhere £165 (members).

The European Journal of Neuroscience. Editor-in-chief R. W. Guillery. Oxford University Press. 12/yr. Europe £260, elsewhere \$495.

NEUROSCIENCE journals are expanding in parallel with the subject but are squeezed by a contracting subscription base. Thus, the dominant force in the increase in the number of journal titles is not reader interest but rather unprecedented author pressure — publish or perish has never been more literal. In addition, the data mountain in the primary literature has created new markets for review journals because reviews, not textbooks, function more and more as the main source of information for non-specialists. But there are already more neuroscience journals than anyone can read or a library can afford. One has to be brutal about recommending new journals. The main problem for each of these new neuroscience journals, although they are targeted to different parts of the markets, is competition with existing titles for manuscripts, readers and, ultimately, subscriptions.

Seminars in the Neurosciences is a review journal organized around a central topic, coordinated by a guest editor. This type of venture overlaps with *Discussions in the Neurosciences*, and individual reviews will overlap with others from a range of other settings, such as *Trends in Neurosciences*, *Annual Reviews of Neuroscience*, *Molecular Neurobiology*, *Brian Research Reviews* and *Current Opinion in Neurobiology*. Given this format, it is difficult to ensure an even quality. For example, an issue on the neurobiology of glia was excellent and original, whereas other issues have suffered a lack of focus and repeat tired themes. For libraries, and to a lesser extent individuals, the journal has been fairly priced, but suffers from its global mission. It would perhaps be better if one could buy individual issues, as can be done with *Discussions in the*

Neurosciences. Nevertheless it is a sensible effort with high standards, although the review format in neurosciences is heavily overworked and I cannot say that the journal is without peers.

Comments on Developmental Neurobiology describes itself as "a journal of critical discussion of the current literature" and has 3–4 short reviews per issue on unrelated topical subjects. The quality of individual reviews is varied but the overall impression, particularly of independent contributions on neural-crest precursor-cell maturation was very good. Successful contributions were readable and well illustrated. This may reflect astute editorial choices of authors who are not over-exposed and are inclined to be more thoughtful and careful in their review. Regrettably, however, the journal suffers from a fatal lack of originality, which together with the low quality of its presentation, makes its price excessive.

Neuroreport represents an interesting idea. The notion is to publish, very rapidly, short papers that make a single point. The brevity of the manuscripts and speed of their appearance outdoes its principal competitors, *Neuroscience Letters* and *Neuroscience Research Communications*. Speed is accomplished by having a panel of paid experts as reviewers, some of whom I expect will not prove as speedy in reviewing as they are in cashing their cheques. Perhaps this is why the journal seems to have a heavy bias towards electrophysiological or behavioural results, with little or no molecular, biochemical or immunochemical work. This may change in time, but a fast-publishing neuroscience journal will always have an uphill battle in convincing a diversity of specialist neuroscientists of its legitimacy. But there is no denying that "a minimum of delay" from submission to publication is appealing. Perhaps *Neuroreport* is the harbinger of the emergence of cadres of paid reviewers, in recognition that reviewing standards suffer when reviewing is a spare-time activity for researchers. The quality of contributions is not as high as the journal aspires to, but I suspect that it will be successful. It can be recommended to libraries but not individuals.

Lastly, the *European Journal of Neuroscience* is the proprietary organ of the European Neuroscience Association, and therefore professes a "European focus" in publishing full-length original papers. Like *Neuroreport*, it has excellent standards of presentation. There is a temptation to view this journal as the inevitable response to its big brother, the *Journal of Neuroscience*, which is linked to the Society for Neuroscience. It seems destined to have an inferiority complex, as the *Journal of Neuroscience* is one of

the foundation journals for the entire subject. At this stage, the *European Journal of Neuroscience* is publishing pedestrian manuscripts — not excellent, not bad. On this basis, it too is not a necessity for either individuals or libraries.

Of these new journals, *Neuroreport* seems to be in the strongest position to make a unique contribution to the neuroscience literature. The others, although worthy attempts, are not as good as their more established counterparts. □

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Blazed trail

Larry W. Swanson

Journal of Neuroendocrinology. Editor-in-chief Stafford Lightman. Oxford University Press. 6/yr. Europe £160, elsewhere \$325 (institutional); members' rates on application to publisher.

EATING, drinking, fighting, sex — their biological underpinnings are all the purview of neuroendocrinology, a hybrid discipline practised by those interested in how the nervous and endocrine systems interact. The idea that humours influence behaviour (and thus the brain) is certainly an old one, but it was not until the 1940s, thanks largely to the work of Geoffrey Harris, that understanding the structure and function of the hypothalamic-pituitary axis became the focus or central theme of what we now call neuroendocrinology.

The number of papers published yearly in this field is truly prodigious and overwhelming; in all likelihood it will increase greatly over the next few years as workers probe mechanisms regulating the expression of genes for hormones and neurotransmitters, as well as for their receptors. In view of this, it is not surprising that another journal has appeared to challenge the relatively venerable *Neuroendocrinology*, *Neuroendocrinology Letters* and other less specialized journals that publish in this field. And I think it is fair to say that within a very short time the quality of research articles published in this new journal is as good as that in its rivals, if not better. Many factors have probably contributed to its success, including the assemblage of a stellar editorial board, its beautiful format as a whole (including a stunning colour graphic illustration for the cover), and the fact that publication delays are short, and costs are low (no page charges and 100 free reprints — thank you Oxford University Press).

On the other hand, subscriptions to the journal are expensive (especially for six issues a year) and raise the general issue of why anyone would want to subscribe to this or any other journal in the field? They are merely expensive archival collections of solid research articles, most of which any one reader will not be interested in. Neuroendocrinology has probably reached the stage where an intellectual focus would be welcome and useful, a place for book reviews, and mini-research reviews,

along with more traditional reviews and original articles, and a place for only the most exciting, original work. Forgive me for repeating an oft-heard sentiment, but the trail blazed by *Cell* for molecular genetics should be followed or explored even further by other leading journals in other disciplines. □

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More of less?

Peter Sheterline

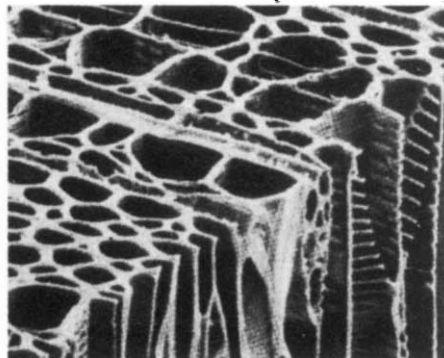
Seminars in Cell Biology. Guest editors. Saunders. 6/yr. US and Canada \$120, elsewhere \$140 (institutional); US and Canada \$75, elsewhere \$95 (personal); US and Canada \$60, elsewhere \$80 (students).

Cell Regulation. Editor-in-chief Erkki Ruoslahti. The American Society for Cell Biology. 12/yr. \$300 (institutional), \$200 (personal), \$130 (members), \$90 (student members).

As a result of some devious logic, I have received two very different types of journal to review contiguously. Both are aimed at cell biologists and both highlight some of the important issues surrounding scientific publishing. *Seminars in Cell Biology* is another venue for reviews of topics of interest to cell biologists. Not so long ago there was something of a dearth of readable, affordable overview journals covering the main systems in cell biology. We needed them then as we do now, partly because the cell-biology view requires at least a nodding acquaintance with cellular bits other than those of direct research interest and a healthy respect for their interrelatedness; ask not for whom the calcium rises; it rises for thee, and partly because, by courtesy of molecular geneticists, the field was and is expanding very quickly. The cry for review material by cell biologists was clearly sufficiently loud to have been heard by almost every scientific publisher. There emerged, consequently, a clutch of cell-biology review journals.

Seminars in follows *Annual Reviews* in, *Current Opinion* in, *Bioessays* and *Trends* in. It appears bimonthly under a guest editor who assembles 5–8 authors to write on a particular topic (chosen by the editorial board?). Volume 1, which covered 1990, consisted of six issues devoted to, respectively: molecular chaperones; sensory adaptation and motor activation in chemotaxis; pattern formation in *Drosophila*; calcium homeostasis in muscle and nonmuscle

cells (what other kinds of cells are there?); membrane cytoskeleton in development (I bet you hadn't thought of that one); and pathways of intracellular proteolysis. There appears to be a fairly loose remit on style and content imposed on both editor and authors. The papers themselves are fairly short (5–10 pages) and are conventional short reviews of recent data. As with any such exercise,



SEM of cut surface of wood ($\times 5,200$). In 1665, Robert Hooke termed similar microscopic units of cork 'cells'. (From *Molecular Cell Biology* by J. Darnell *et al.*, published by Scientific American Books.)

some of the topics are covered very well, some not so well. There is no attempt to provide a complete reference list, but the references are relatively up-to-date; the first 1990 references, for example appeared in the August 1990 issue.

The journal is very conventional in comparison with the highly structured approach of the *Current Opinion* series, one of its direct competitors. *Current Opinion* volumes contain a relatively complete annual bibliography in identified topic areas and a promise to revisit sites of scientific interest annually. In my view, the *Current Opinion* series, although far from perfect, heralds a welcome new professional relationship between publisher and scientist who collaborate to produce a valuable resource. *Seminars* is not hampered by any such objectives, nor by the commitment to cover key topics annually. On balance, I do not think that the journal offers anything that is either particularly well conceived or executed and I cannot see why many scientists would wish to commit their scarce funds to a review journal whose content is entirely unpredictable,

as can be seen from the 1990 issues. It is just another publication to add to the burgeoning list that uses the energies and resources of both authors and publishers to no great effect.

Cell Regulation is a strictly research publication dedicated to this field. I cannot think off-hand of many things in cells that do not involve regulation, so the title has neither restricted the scope of papers that can be submitted to the journal nor defined a clear area for aficionados. It is a well-produced journal of the *Cell* ilk, but contains papers that are more reminiscent of the *Journal of Cell Biology*. One can't help thinking of it as simply 'Son of *JCB*'.

The papers are of high quality, and, according to the blurb, the journal uses "an innovative routing system for manuscripts" (completely eliminating referees?) to allow publication of papers submitted by the beginning of one month to be published in the issue four months on. Not bad, but not unique. A quick check on whether the promises were sustained by the actuality revealed that, generally, they were. In the December 1990 issue, for example, all papers were submitted between 28 July and 6 September, most being submitted in August. There are about seven papers per issue, which makes me wonder why the American Society for Cell Biology does not simply increase the size of the *Journal of Cell Biology* by a few per cent and save some trees. I cannot see why libraries or individuals should rush out and buy *Cell Regulation*, which would seem to be an unnecessary clone of its parent.

How do scientists and libraries best exert customer pressure on publishers and potential journal editors? Most libraries cannot now afford to continue complete sets of all cell-biology journals, let alone life-science journals; it is important, therefore, that libraries establish well-thought-out systems for rigorously assessing journal quality and usage, and adapt their subscriptions accordingly. In some senses, the more ruthless the libraries' quality assessments, the more pressure there will be on publishers to consider more carefully their objectives. There is not much dissension over which are the valuable core journals; these are ultimately the result of who publishes what in them (as it should be). *Cell Regulation* may well flourish because of its heavyweight patron. Perhaps the entrance to all publishing offices should be inscribed "Less but better", as should those of the funding bodies, whose insatiable paper-lust probably drives the entire machine. □

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Following the fashion

Michael Akam

Seminars in Developmental Biology. Guest editors. Saunders. 6/yr. US \$130, elsewhere, \$155 (institutional); US \$81, elsewhere \$102 (industrial); US \$63, elsewhere \$84 (personal).

SINGLE-topic guest-edited volumes are the flavour of the month among publishers of new review journals. This format suits developmental biology well, for many areas of the subject are not sufficiently coherent to be encapsulated in single pre-digested mini-reviews. This is well illustrated by the volume of *Seminars in Developmental Biology* that examines cell behaviour during morphogenesis. Edited by Raymond Keller and Diane Fristrom, the seven reviews it contains could not sensibly be condensed into a single gobbit, but together provide a rich taste of the field for any student or researcher with a little time spare to chew the cud.

The editorial board of the series has only nine members — small enough to suggest that each takes some individual responsibility for its content. So far they have done a good job, picking for the first year a mix of topics, some fashionably predictable (cell interactions in development), others less so (heterochronic changes in development). There is room in these volumes for a satisfying diversity of approach to each topic, ensuring that most issues will bring something new, even to those familiar with the field.

These volumes are at their best when they provide a coherent view of a well-defined subject area. An example is the issue edited by Claudio Stern on the evolution of segmental patterns. This volume explains why interest in segmentation has revived and outlines the outstanding questions. Some articles are anatomical, others molecular. One is explicitly historical. I thought the inclusion of the latter an excellent idea, and a precedent that might be repeated for other topics.

The appearance of the journal does little to grab or hold the attention of the casual browser. The stark black and orange design on the cover dominates a small inset panel that illustrates the subject of each volume. What a shame, when new techniques in developmental biology are producing such striking visual images. Inside, the quality of reproduction is just sufficient to do justice to most micrographs, although not all of the half-tones fare well. The exclusive use of black-and-white illustration may soon appear dated in comparison with

the increasing exploitation of colour in other review series.

But besides these niggles on presentation, the journal has made an impressive start and could help to integrate the various branches of developmental biology, including those that extend beyond the current concerns of cell and molecular biologists. □

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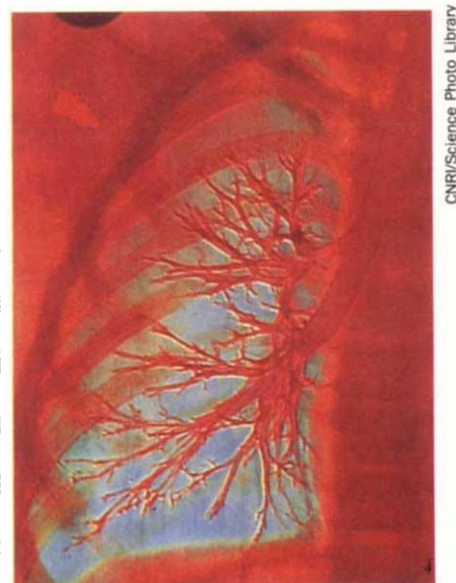
An inspiration?

P. T. Peachell

American Journal of Physiology: Lung Cellular and Molecular Physiology. Editor-in-chief D. J. Massaro. The American Physiological Society. 12/yr. US \$120, Canada \$140, elsewhere \$151 (institutional); US \$95, Canada \$113, elsewhere \$125 (personal).

It is with increasing frequency that established journals identify either new or expanding areas that merit special attention, sometimes leading to the spawning of a new journal such as this one. Since August 1989 the journal has been available both as a subsection of its parent journal, the *American Journal of Physiology*, and on its own. The title is somewhat unwieldy and confusing, because the journal could be misinterpreted as subserving two discrete topics. Be that as it may, the aim of the journal is to encompass, more or less, all aspects of lung physiology.

Each issue contains invited reviews, commentaries, papers on original work and, occasionally, a historical perspective. The reviews are usually related to some aspect of lung physiology, whether at the molecular or cellular level, although on occasion the reviews are far more generalized and would be amenable to a much wider audience. Moreover, a considerable proportion of the articles that appear in the journal would sit comfortably in a journal with a pharmacological slant. As one would expect from the offspring of a journal of some standing, the material is well written and the articles are mostly informative without necessarily being spell-binding. The editors have laudably devoted a large proportion of the articles to molecular topics, but the coverage of cellular aspects seems to suffer an imbalance towards certain cell types. Of course, this may simply reflect the bias of those who submit material to the journal, but on this account the journal's appeal might suffer. This also means that individuals who might benefit hugely from



CNRI/Science Photo Library

False-colour arteriograph of a normal lung.

reading the journal (such as readers of the *American Review of Respiratory Disease*), may be discouraged from doing so. These caveats apart, the journal is informative and is a welcome addition for an area of research that has a growing following. □

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Stimulating start

William Cushley

Cytokine. Editors Scott Durum and Gordon Duff. Saunders. 6/yr. US \$275, elsewhere \$300 (institutional); US \$125, elsewhere \$150 (industrial); US \$90, elsewhere \$116 (personal).

RESEARCH into the biological activities of cytokines continues to expand at an apparently exponential rate. The appearance of a periodical devoted to this area of endeavour was, therefore, entirely predictable. Is there a need for a specialist journal in the area? I think that there is, given that many good cytokine papers currently find themselves in subsections of journals devoted to more general areas.

The title of the journal is broad, suggesting that contributions dealing with a variety of cytokine-related processes will be welcomed. For the most part, the journal lives up to its name, presenting reports on a wide selection of topics. These range from routine papers detailing *in vitro* effects of individual cytokines, to reports in the currently fashionable areas of cytokine receptors and

molecular biology of cellular responses to cytokine stimulation. An investigation of the implications of interleukin-6 levels in the amniotic fluid of pregnant women at parturition provides a good example of a novel report from the clinical front line. In addition to the full papers presenting complete pieces of work, a positive feature of *Cytokine* is the inclusion of brief reports of less than four pages presenting novel findings in a succinct fashion. Most of the papers are of good quality, being well written and informative. The policy of having occasional reviews is a good one, as it will allow readers to keep up with advances in understanding of the properties of cytokines other than those of their own immediate interest.

The breadth of coverage is in keeping with the editors' stated wish to attract contributions to *Cytokine* from sources other than immunology and haematology research groups. But a nagging feature of the issues provided for review was that more than one-third of the papers were concerned with interleukin-1 in some way; reports dealing with

interleukin-6 were also in abundance. Although this might reflect the inherent pleiotropy of these two cytokines, it could signal a trend towards *Cytokine* becoming a journal aimed largely at those interested in interleukin-1 and -6, which would be a great pity. I am sure that a more balanced coverage of all the principal cytokines will be achieved as *Cytokine* becomes better known and recognized by workers in the field as a whole.

The quality of production is very high. In particular, the reproduction of graphical material, tissue sections and autoradiographs is excellent. The bold blue cover of the journal, complete with original abstract painting, is doubtlessly designed to ensure the journal stands out on the library shelf. The challenge for the editors will be to keep up the high quality of papers and to ensure that the contents are as eye-catching as the cover. □

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Something for all families

Robin A. Weiss

Seminars in Virology. Guest editors. Saunders. 6/yr. US \$120, elsewhere \$145 (institutional); US \$64, elsewhere \$85 (industrial); US \$48, elsewhere \$69 (personal).

SEMINARS in Virology is a recent addition to the Saunders series, commencing publication in January 1990. Each issue comprises 6–8 short reviews on related topics coordinated by a distinguished guest editor chosen for the particular field. An attractive feature is that most issues treat an aspect of virology that spans different virus families. Thus the first issue dealt with modern approaches to vaccines (editor, F. Brown), the fourth with mechanisms of viral pathogenicity, including plant and insect viruses (editor, R. S. Fujinami), and the sixth with virus structure (editor, J. Hogle). Some issues cover a particular

virus group, but even then there is breadth of treatment.

The individual review articles cover the authors' special interests, and the choice of chapters in each issue does not attempt to be comprehensive. This adds spice to the articles, as they are written by enthusiasts. In each issue I found several interesting and illuminating reviews. By and large, the articles are well illustrated and successfully encapsulate current knowledge and ideas in their respective fields.

There are few other review journals in virology and this one is a welcome addition to the field. Whether the topics and treatment will remain as lively and tidy as they were in the first nine issues remains to be seen, but I can recommend the series to virologists from graduate students onwards. It is doubtful whether the journal as a series will attract much interest among nonvirologists, but individual issues should. Viruses have been at the forefront of numerous basic discoveries in cell and molecular biology and deserve attention beyond the field of infection. □

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Special agents

T.A. Connors

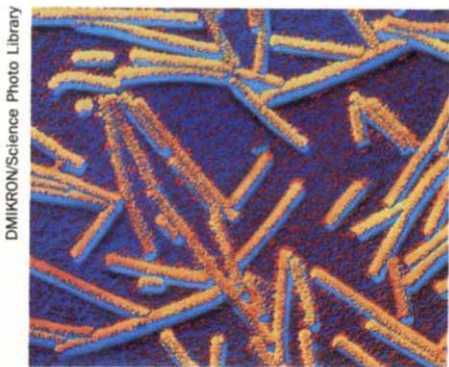
Anti-Cancer Drugs: An International Journal on Anti-Cancer Agents. Editor Mels Sluysers. Rapid Communications of Oxford. 6/yr. £222, \$399.

THE ultimate aim of every cancer chemotherapist is to design the perfect anticancer agent — a chemical that will cure patients of a cancer at a dose that has no side-effects. It was rather surprising, therefore, to read in the aims and scope of the journal that "the editors recognised the need to encourage research on non-toxic cancer agents". Assuming they mean anticancer agents, then chemotherapists have been attempting to do this for years and are hardly in need of encouragement. Toxicity is not deliberately designed into the molecule but is rather the sad consequence of being unable to discover truly selective anticancer agents. Reading further, it appears that the editor is distinguishing between cytotoxic agents and biological-response modifiers, the former being classed as toxic and the latter as non-toxic. Whether biological-response modifiers can be considered to be a nontoxic class of agents is arguable, but one nevertheless follows the line of thinking.

All things considered, the journal has got off to a good start, no doubt assisted by its rapid publication policy which aims to peer review and publish acceptable papers within 30–60 days. Wisely, and presumably to guard against a possibly low level of submission of original papers in the early editions, a number of review papers have been commissioned. These are wide-ranging and uniformly good, some such as Judson's on anthra-pyrazoles being very topical.

In the editor's own words, the research papers comprise a plethora of subjects, which is a little unkind, since plethora is usually defined as an overfullness or unhealthy excess. Certainly the subjects covered are wide-ranging and deal with anticancer drugs in the broadest possible sense. It is to be hoped that some of the 'research' articles that are case reports of toxicity will not feature prominently in the future, nor articles that describe the effects of nitrogen mustards on transplanted tumours, bearing in mind that by 1960 more than 2,000 nitrogen mustards had been synthesized and shown to be active against transplanted tumours. I assume that the unimaginative papers will eventually be replaced by more original articles, of which there is already some evidence.

The rapid review system appears to be working well, with all published papers



Tobacco mosaic virus ($\times 72,600$), the cause of leaf mosaic disease in tobacco plants.

being refereed within 45 days with an average of around a fortnight. The most rapid acceptance was the paper on the Caret trial, which was approved on the day it was submitted, or even perhaps the paper by Prasad and Arjun, which had no receipt or acceptance date so presumably was accepted in no time at all.

Scientists continually bemoan the plethora (if that is indeed the right word) of new journals, but it seems inevitable that more and more will be published. If so, then one hopes they will be as well presented as *Anti-Cancer Drugs*, which should develop into a respectable research journal. □

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Good exposure

L. J. Kinlen

Cancer Causes and Control: An International Journal of Studies of Cancer in Human Populations. Editor B. MacMahon. *Rapid Communications of Oxford.* 6/yr. £222, \$399.

CANCER kills an appreciable proportion of the world's population (in North America and Europe, around 1 in 5) and many more suffer from its curable forms. The study of its causes in human populations (epidemiology) is therefore of considerable importance. *Cancer Causes and Control* was launched in July 1990 under the editorship of the distinguished epidemiologist Brian MacMahon.

As the opening editorial reminds us, epidemiology has already made great strides in indicating the roles played in human cancer by tobacco, asbestos, infection with hepatitis B virus, sunlight, ionizing radiation and certain chemicals. Indeed, if exposure to these were eliminated (or reduced in the case of ultraviolet light), cancer rates would probably be reduced by a third or even more.

Traditionally, papers in the field of cancer epidemiology have appeared in general medical, cancer or epidemiological journals. There is no doubt that the specialized nature of many papers makes them unsuited to a general readership, whereas cancer journals are mainly devoted to laboratory or clinical studies. *Cancer Causes and Control* has the distinction of being the first journal to be devoted exclusively to cancer epidemiology. Emphasis is to be placed on speed of publication — an aim so far achieved, all papers having been published within two months of acceptance. The coverage of subjects is wide, but contributions in

the first two volumes have concerned one of the most important current problems in cancer causation — whether dietary fat causes cancer of the breast and other organs. I cannot think of another journal that would allow so extensive an airing of differing views. This and the speed of publication augur well for its future, although it is expensive. □

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Bright future

D. P. Lane

Seminars in Cancer Biology. Guest editors. Saunders. 6/yr. US \$130, elsewhere \$155 (institutional); US \$130, elsewhere \$102 (industrial); US \$63, elsewhere \$84 (personal).

WHY do people agree to write reviews? I suppose the full range of human emotions are involved. At the more base level they are flattered to be asked, the deadline seems about two lifetimes away and it gives them a good opportunity to rewrite history, emphasizing the unique importance of their own contribution. It might look good on their curriculum vitae and do wonders for their ranking in the Science Citation Index. They might even get paid as well. Nobler motives might include a desire to try and pull the field together and make it more comprehensible to others. At best the author can bring fresh insight to an area that will colour future research.

Why do people read reviews? The need comes from the vast size of the primary literature, from its bias, and from a need to avoid arcane acronyms. Move slightly out of your own field and the primary literature can become impenetrable. Certainly it becomes very difficult to make a critical judgement about rival claims. A striking example of this is the recent attempts of one and all to understand the amusing serology of idiotypes in the Weaver *et al.* paper at the centre of the Baltimore affair. Good reviews can bridge the gap; they need to be independent, up-to-date and critical. At the technical level they need to be short and provide an entry into the relevant primary literature.

Seminars in Cancer Biology is a much better series than I expected. There are several established rivals in this field but the series has found a niche because of its format. Each issue uses a new guest editor, an expert in the field, and covers a specific topic. The reviews are short and show signs of good editing. Thank-

fully references are given in full. The coordination between volumes is generally good, though Fos and Jun, for example, are reviewed three times in the six volumes I looked at, exceeding even my great admiration for these proteins.

So if the idea is valid and the organization good, what about the reviews themselves? I looked at two volumes in my field, that on transcription factors edited by Nick Jones and that on nuclear oncogenes edited by Gerard Evan. Both of these were really very good. The reviews were up-to-date and pitched, I thought, at just the right level. Generally they were written by well-known people in the field and with some authority. I would have liked to see a bit more direct talking when discrepancies in the field were discussed, but overall the standard was high. The other volumes, more peripheral to my own work, were also encouraging. Those on antibodies, on the epidermal-growth-factor receptor family and on proteases, were at the right level and were well coordinated.

There is a need for this kind of series, as it forms a bridge between the exhaustive traditional review and the kind of summary that marks out the Current Biology series. As more of molecular oncology drifts towards the real world of treatment, this series will prove helpful to keep us all up-to-date. If the present standard and momentum can be maintained then it has a bright future. □

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In company

Michael T. Lotze

Biotechnology Therapeutics. Editors Steven Gillis and Arthur Ammann. Dekker. 4/yr. \$225 (institutional); \$117.50 (personal).

ONE of the secrets to the rapid development of interleukin-2 biology was the availability of a rapid, extremely sensitive bioassay using the CTLL line identified by Steven Gillis. This approach to identifying biological activities, developing assays and then cloning the genes encoding them has recently been duplicated with many other growth factors and cytokines and represents one of Gillis's lasting legacies. I remember running into him at a conference almost a dozen years ago after he had made the rounds of traditional academia at Dartmouth, Memorial Sloan-Kettering and the Fred Hutchinson Cancer Center, when he indicated that he was inclined to start a company to develop biotechnology further. He, together with Larry

Lachman and Chris Henney, provided the scientific drive and attraction for a highly productive and creative stable of biologists, protein chemists and molecular biologists at Immunex. Industry is now the unabashed centre for many of the rapid advances in cytokine biology. Certainly the rapid identification of cytokines and their receptors, their cloning and ultimately production for clinical trials has largely become the domain of Immunex and others of their ilk. Rapid proliferation of biotechnology companies in the United States has become the envy and model for similar start-up companies overseas and it is likely that such outfits capable of generating sufficient funds for applied research and ultimately their application clinically will be the main province of biotechnology. It should not be surprising then that Gillis and coeditor Ammann from Genentech should have chosen to develop another forum for their efforts.

Thus we have this new journal. The assembled editorial facesheet reads like a *Who's Who* of biotechnology with 10 out of the 24 individuals representing companies such as Immunex, Genentech, Genetics Institute, Cetus Corporation, DNAX Research Institute and Amgen. The need to have a specialized forum however, as initially conceived by the editors for a single source of articles evolving around biotechnology, is belied by the concurrent rapid development of its competitors. Joining the *Journal of Immunotherapy* are other new journals, including *Biotherapy* and *Molecular Biotherapy* (reviewed in *Nature* last year) *European Cytokine Network*, *Cytokine* (see page 465) and *Human Gene Therapy*.

Out of 21 articles in the first four issues, 16 deal with growth factors and cytokines and 7 are from either biotechnology firms exclusively, or coauthored by an individual representing one of the firms. The journal consists largely of reviews and occasional original articles, with a single issue of the four focusing on summaries from an Illinois Cancer Council Symposium. Most of them are clear and well written but I suspect are still not yet the premiere groundbreaking articles (I wrote one of them) generated within or without the biotechnology industry. Although the publisher indicates that contributions to the journal are published free of charge, the cost to individuals at about \$0.50 per page is about double that of the *Journal of Immunotherapy*.

As any good drug-house knows, one has to develop 10 new drugs for one wildly successful one to make it in the market-place. The same may be true with the recent proliferation of biotechnology journals. This particular one seems to be most clearly

spawned of itself, and to its credit, is the only one to have a representative from the distaff side as an associate editor. Susanna Cunningham-Rundles indicates that the main focus for the journal is to publish the best-quality manuscripts possible, but also early and not yet complete stories of the application of cytokines, as well as important negative studies.

With 30,000–100,000 genes within the human genome having evolved to potentially find themselves listed on the spreadsheet of a biotechnology firm, and only a fraction of them developed, the future is bright indeed for these products to become ultimately the mainstay of

clinical therapeutics. Certainly with six hundred billion dollars spent in the United States alone for health care in 1990, and an anticipated 1.5 trillion by the year 2,000, a large potential market for such products would seem to exist. The question of the viability of biotechnology journals and the industries that generated them will largely be dependent on our ability to afford them and their ability to replace other costly technologies (and journals). □

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TG or not TG?

Steve S. Sommer

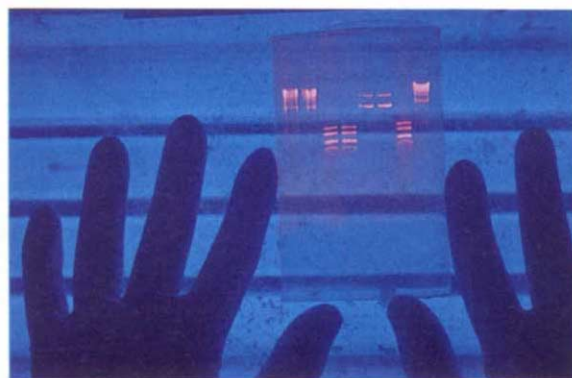
DNA Sequence: The Journal of DNA Sequencing and Mapping. Editor Bart Barrell. *Harwood Academic*. 6/vol. *US and Canada* \$293, *elsewhere* Dfl. 644.

WE are nice enough to lawyers, used-car salesmen and 'sequence jocks' when we need them, but deep down we feel morally superior. Sure, sequencers can still get a paper into *Nature* if the God of sequence homology bestows an unexpected and revealing pattern, but in general, sequencing is now routine and of little intellectual challenge. Hence, increasingly, established journals in the biological sciences either do not publish sequencing papers or restrict the length of those that are published, particularly when there are no additional experimental data.

If the above strikes a responsive chord, read no further. If, on the other hand, you are impressed with the ever increasing number of insights provided by an educated analysis of sequences, *DNA Sequence* may be for you. This new journal publishes mapping data useful to future DNA sequencing, DNA sequence data in full, new sequencing procedures and allied techniques, computer methods to analyse DNA sequences, and compilations of sequence motifs. Emphasis is given to papers that include methodology and collation of sequences. High-quality reviews are invited. The journal provides a forum for the exchange of research experience and advice through a 'Letters to the editor' section. *DNA Sequence* is based at the Medical Research Council's Laboratory of Molecular Biology in Cambridge, United Kingdom. Bart Barrell and

John Walker are the editor-in-chief and deputy editor, respectively. John Sulston and Alan Bankie are the mapping and sequence method editors, respectively. The US editor is Clyde Hutchinson III at the University of North Carolina.

The papers in the first four issues were diverse and a number of prominent laboratories were represented. A partial list of titles is perhaps the best way to give the reader a sense of the contents: 'A dictionary of transcription control sequences'; 'Sequencing with the large fragment of DNA polymerase I from *Bacillus stearothermophilus*'; 'Alterna-



DNA banding pattern in an electrophoresis gel. The pattern in pink fluorescence is revealed under ultraviolet light.

tively spliced *Hox-1.7* transcripts encode different protein products'; and 'Mapping DNA by stochastic relaxation: schedule for optional annealing'.

DNA Sequence may well serve the needs of a growing number of scientists who want a forum for all aspects of sequencing including methodology development, sequence analysis and compilation of sequence motifs. I sense that this journal will do well and I anticipate that it will soon spawn a companion journal, *RNA Sequence*, which in turn will spawn *Codon* and *Untranslated Regions*. Such is the nature of progress. □

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Peter Menzel/Science Photo Library

Modus operandi

Charles S. Craik

Methods: A Companion to Methods in Enzymology. Editors-in-chief John N. Abelson and Melvin I. Simon. *Academic*. 6/yr. US and Canada \$132, UK £116, elsewhere \$168 (institutional); US and Canada \$86, UK £76, elsewhere \$110 (personal).

Protein Expression and Purification. Editor-in-chief Owen W. Griffith. *Academic*. 6/yr. US and Canada \$140, UK £114, elsewhere \$164 (institutional); US and Canada \$70, UK £61, elsewhere \$88 (personal).

In the current atmosphere of a virtual deluge of new journals with a disturbing number focused at separating the reader from the primary work, two new ones appear on the scene that provide a refreshing breath of data. You will not find any big picture stuff here, but a wonderfully dizzying array of details. These details may bore the 'movers and shakers' of the scientific world but will bring warm smiles to research directors, postdoctoral fellows and graduate students who actually have to carry out the grand experiments. How many of us have spent long hours working out the finer points of a method that could save someone else considerable time, only to have it relegated to a scant few sentences in 'Materials and Methods' or, the ultimate injustice to all involved, denoted as 'unpublished results' owing to page limitations?

When a particular technology develops into an established set of protocols that can benefit a general audience, places exist for archiving this information. Thirty-six years ago, *Methods in Enzymology* was established to assemble various tried and true protocols into comprehensive volumes that could be referenced in much the same way one consults an encyclopaedia. Efforts were made to focus on new techniques and to make the protocols accessible to the expert as well as the novice. The efforts have been well spent and the resulting oft-consulted volumes are prominent in any respectable biochemical library. But their sheer size and definitiveness prevent them from keeping abreast of cutting-edge developments in rapidly changing fields. The current pace of technological development requires a different forum for describing the conventions of a hot new area — a sort of mini *Methods in Enzymology* that provides a concise, up-to-date, in-depth description of exciting new technologies.

In August 1990, the first issue of *Methods: A Companion to Methods in Enzymology* was published. The issue

focused on protein and nucleic-acid crystallization, a topic of increasing importance but of limited recognition. Subsequent issues have or will deal with pulsed-field electrophoresis, covalent modification of proteins by lipids, new applications of the polymerase chain reaction (PCR), DNA sequencing and two-dimensional gel separation of proteins. These subjects involve rapidly developing technologies that require frequent methodological updates.

Each issue of *Methods* has a coordinating guest editor that assembles the works of various contributing authors who are actively participating in the topic of interest. These editors may in-

cated souls who wish to enter into the realm of crystal growth.

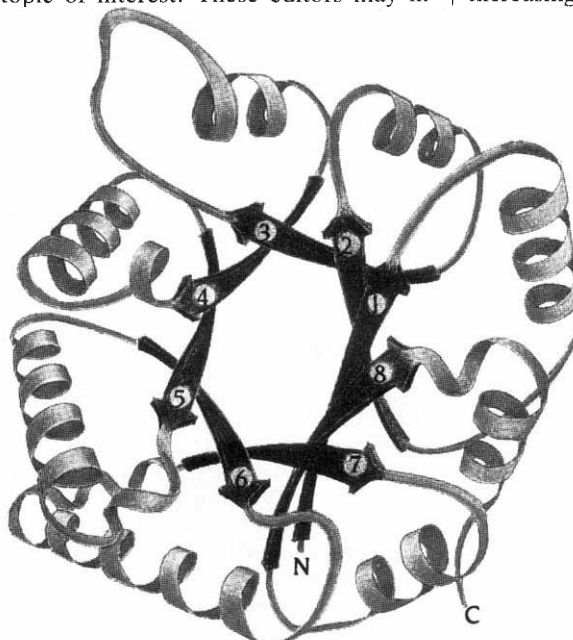
The issue on pulsed-field gel electrophoresis is a useful introduction to the proliferation of instrumentation for manipulating DNA in the megabase size range, which is playing a critical role in modern biology. Instrumentation such as field-inversion gel electrophoresis, rotating gel electrophoresis, transverse alternating-field electrophoresis, and contour-clamped homogeneous electric-field electrophoresis is described in theory and in practice. The issue on covalent modification of proteins by lipids is extremely timely considering the increasing abundance of reports describ-

ing various new forms of these modifications, many of which are providing insights into the functional significance of the modified protein.

The issue on PCR is surprisingly fresh considering the number of monographs recently published on the topic that provide protocols and discuss diverse applications of the technique. Simply comparing several of the protocols in the *Methods* issue to the previously published books, as well as to the protocols in the 1987 article on PCR in *Methods in Enzymology*, shows how rapidly the field is changing. Immediate access to the detailed changes is clearly invaluable to someone working in the field.

A satisfying aspect of each issue is the common theme of the various contributions. Trends can often be recognized in such a concentrated source of related articles that provide confidence in particular protocols. The number of articles that are worth reading per issue adds an extra boost for the researcher attempting to overcome the activation energy for embarking on a particular technological endeavour. Clearly, the difficult task for the organizing editors will be to maintain the current level of sophistication and topical relevance.

A different type, but nonetheless useful 'how to' journal is *Protein Expression and Purification*. Here the various articles in a given issue are related only in that they each involve expression and/or purification of proteins. Articles and reviews focus on new or considerably improved procedures for producing proteins of reagent quantity and quality. Novel expression vectors and hosts are described as well as the use of these systems to increase yield, stability, solu-



Pure form — closed barrel of α/β domains of the enzyme triosephosphate isomerase. (From *Introduction to Protein Structure* by C. Branden and J. Tooze, published by Garland.)

clude a piece on their own work and/or provide an introduction or general overview. They are primarily responsible, however, for organizing a cohesive collection of articles that serves as a distillation of unifying ideas and specific protocols from the literature. The results so far have been promising. The issue on crystallization of macromolecules is required reading for the structural molecular biologist. Attempts are being made to make the processes of protein and nucleic-acid crystallization more certain and reliable — to change this art into a science.

It is exhilarating for a biochemist to realize that any macromolecule can be crystallized if the correct conditions can be found. The logical approaches that can be applied to achieve this crystallinity, as well as some of the creative approaches involving microgravity conditions on the space shuttle, show that the efforts need not be tedious, fruitless or mundane. The articles help provide a perspective and impetus for those dedi-

bility or activity of a given protein. Alternative or more efficient methods for fractionating and purifying the proteins are clearly detailed and many of them are useful to someone struggling with an expression system or purification scheme of their own. It is refreshing to see the resurgence of interest in the practical and theoretical aspects of protein production and purification.

It is often forgotten that much of the original impetus for developing recombinant cloning technologies was to overcome the limiting reagents problem. Many of the proteins such as repressors, activators, hormones and processing enzymes that were of paramount importance to investigators in the early 1970s were quite rare and in extremely limited supply. It was mandatory to clone and overexpress the targets in order to study them. But since it became much easier (and in some people's opinion, more fun) to clone, sequence and characterize a gene than to analyse the biophysical nature of the protein encoded by the gene, many of the original goals were superseded. It is therefore pleasing to see individuals on the editorial board who love the methodology-oriented practice of protein purification itself. Robert Scopes, one such member, stated in the preface to his book *Protein Purification: Principles and Practice*, "The fascination of isolating a reasonably pure enzyme from a complex natural soup of protein remains with me; as a challenge and an academic exercise I still spend much time on enzyme purification even when I have no real use for the final product!"

In today's world of new start-up ventures in everything from journals to biotechnology companies, one must take a cautious wait-and-see attitude before investing one's precious manuscript or life's savings. The future seems bright for these two journals, but the competition for quality manuscripts will be stiff. I admonish the members of the editorial boards of the two journals to provide a first-rate theatre for the auditioning of details. Performers, such as the meticulous graduate student or the post-doctoral fellow, should be invited to describe their methods for someone to repeat on the first attempt. The biochemist whose publication list boasts several papers in either of these journals would then be the one to look out for. □

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Electrifying separations

David C. Schwartz

Applied and Theoretical Electrophoresis. Editor-in-chief Carl. R. Merrill. Macmillan. 6/yr. £80, \$140.

SINCE the time of Tiselius, electrophoresis has attracted an interesting assortment of scientists united in their quest for either putting their favourite purified molecules into bottles or understanding the physical mechanisms behind the electrophoretic processes responsible for these separations, or possibly both. As can be expected, electrophoresis research is very interdisciplinary with its breadth covering physicists' modelling systems on Cray supercomputers, to clinicians examining spinal fluid for disease. It is not surprising that Linus Pauling helped launch molecular biology, in part, with his remarkable electrophoretic analysis of globin mutations responsible for sickle-cell anaemia more than four decades ago. Since then, electrophoresis-based analysis has played a pivotal role in the biological sciences. It is this blend of physics and biology that makes *Applied and Theoretical Electrophoresis* a valuable journal to physically oriented scientists searching for

new physical phenomena to investigate as well as to biologists requiring new methodologies to solve difficult problems in molecular analysis. The journal is, of course, also of great interest to researchers working directly in this field.

Most of the issues have a nice blend of articles covering computer analysis of data, work to characterize and find new separation matrices, as well as the expected array of articles examining a myriad of electrophoresis separation mechanisms. Biochemists and molecular biologists will find that articles are evenly distributed between protein- and nucleic acid-based separations. New electrophoretic techniques are also covered along with numerous broadly useful applications — this will endear the journal to scientists who are not really interested in electrophoresis. Often more specialized electrophoresis journals publish applications with only limited audience appeal. It is also gratifying to see that extensive materials and methods sections accompany most articles, thereby enabling researchers to readily try out new techniques.

Applied and Theoretical Electrophoresis is a journal that should be consumed by most biologists as well as interested physical scientists. □

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Going against the stream

Barry L. Karger

Bioseparation: International Journal of Separation Science in Biotechnology. Editor-in-chief Tony Atkinson. Kluwer. 6/yr. Dfl. 506, \$257, £172 (institutional); Dfl. 285, \$150.50, £97 (personal).

Is this one necessary? Journals seem to increase in number by leaps and bounds, and the field of separation science already has many, including *Journal of Chromatography*, *Preparative Chromatography*, *LC/GC* and *Separation Science and Technology*. But editors of *Bioseparation* argue that separation techniques for downstream processing in biotechnology are too spread out among other journals (including those of chemical engineering), and aim to remedy this situation.

After examining the journal I agree that the emphasis on downstream processing is warranted. Furthermore, the quality of the papers is most pleasing. In general, they seem to be very useful, well thought out and well presented. It is fair to say that some of them would be overlooked if they were to appear in a more diverse journal.

Other important aims of the journal are to publish both reviews and issues devoted to special topics. The December 1990 issue dealt with aqueous two-phase separation systems. Such issues have real value in that a great deal of current information on a particular topic can be assembled in one place. This format has also been well incorporated into other journals.

A good start has been made in establishing a high-quality journal for downstream processing in biotechnology. I hope that there will be increased emphasis on high-performance separations in preparative-scale chromatography as well as that placed on preparative-scale electrophoresis. These two topics will clearly evolve as the journal expands its coverage. In general, the journal is recommended as a useful source for downstream processing.

The editorial board consists predominantly of engineers rather than chemists and, therefore, large-scale separations feature greatly in the journal. Will the editors also become interested in process analysis? □

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Intelligent looks

Graham A. Dunn

Journal of Computer-Assisted Microscopy. Editor-in-chief John C. Russ. *Ple-num*. 4/yr. US and Canada \$115; elsewhere \$135.

THERE is hardly a human endeavour that is not computer-assisted these days and so perhaps the title of this new journal is intended to warn off 'computerphobes' rather than to suggest a highly specialized area of microscopy. Computers are certainly playing a central part in the revolution that is happening in microscopy: sophisticated image processing is helping to erode the old 'fundamental' limits on the type of information that can be gathered optically; many innovations at the forefront of microscope technology are entirely dependent on digital processing and even routine data from microscopes are now rarely interpreted or quantified without some form of computation being used.

The main role of computers in this revolution has so far been rather mundane. Most of the image processing and analysis is commonplace and the computer control of microscope functions is still largely rudimentary. But computers are already proving to be an essential adjunct to certain new types of microscopy and must soon be regarded as part of the microscope, as indispensable as the optics. The enormous quantity of information that can be generated by scanning confocal microscopy, of living material, for example, can only be stored and adequately interpreted through digital processing. A vision of the future in which we can effortlessly explore the dynamic four-dimensional structure of a living, developing embryo, changing our direction of view at will and moving back and forth in both time and space, is probably now more contingent on progress in software and in digital processing than on progress in optical technology.

This new journal is timely because microscopists must now acquire expertise in computing to avoid becoming slaves to manufacturers' hidden software. No analysis of scientific data should be acceptable for publication if the investigator is ignorant of the algorithms used. The first few issues have done well to bypass the more routine aspects of image processing and storage and have concentrated on computer applications that do not normally appear in books on the latest microscope techniques. The main emphasis seems to be on data reduction and analysis and there

is a surprising wealth of information on what may be loosely described as metrology and stereometry: harmonic shape analysis, surface curvature analysis, adjacency measures in tessellations, and so on. These are obviously the main areas of interest of the editor-in-chief, who has already contributed no less than 12 mathematically sophisticated articles. Taken as a whole, the articles cover a ragbag of topics from correction of piezoelectric creep in scanning tunnelling microscopy to goniospectrophotometry

of meat. The only consistent theme that I could detect is that the articles are sure to be enjoyed by computer buffs and by microscope innovators alike. One of the manufacturers of scientific equipment assures us that the age of really intelligent microscopes has arrived; perhaps the new journal will provide a forum for them to communicate with each other. □

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Thinking big

R. J. P. Williams

Journal of Biomolecular NMR. Editor-in-chief Kurt Wüthrich. *ESCOM*. 6/yr. Dfl. 600, \$310 (institutional); Dfl. 190, \$95 (personal).

A JOURNAL of this title has to be largely concerned with biological polymers because NMR characterization of smaller molecules, whether they be biological, organic or inorganic, is common ground for many chemistry journals and is fairly routine. No new organic molecule, even fulvarenes, can now be described in an acceptable manner without an NMR spectrum. So what is special about large-molecule NMR? It is very difficult to analyse with great confidence NMR spectra of molecules that exceed a relative molecular mass of about 4,000 (4K) and that are not rigid, and the problem becomes horrendous for molecules of 50K or more. This journal is concerned with these problems for large biological polymers.

Although NMR can give a good picture of the structure of the more rigid elements of regular secondary features of, for example, a protein or a DNA fragment in solution, we do not really know how good the pictures are of irregular strands or side chains. The problem has two parts: can the methods used define rigid structure, if any, accurately; and can any nonrigid structure be determined at all in solution? The papers in the first issue reveal these problems. There are three papers on assignment procedures, that is operational problems before structural analysis, three on structural study and three on dynamics. The trend is to ever-more sophistication in assignment technique and in theory. Clearly it is useful in one sense to discuss these problems and the technical developments all in one place, but could not the problems have been managed under the *Journal of Magnetic Resonance*? Many libraries will not subscribe to yet another spectroscopy journal, especially with the increase in the size of existing journals.

Judging from the quality of the editorial board and the first issue, good papers will be generated for at least a short while. But will the journal last over a longer period? Will it publish NMR studies of biomolecules that have been analysed by crystal-structure studies? Crystallographers often criticize 'structures' deduced by NMR methods while forgetting the complementary nature of the knowledge gained, especially on chemical interactions and dynamics in solution. And is a separate journal on NMR spectroscopy of biopolymers really warranted for the study of biological structures? Would not existing combinations of journals on NMR on the one hand and biological structures on the other serve better? And at this price, the journal is very discouraging except for rich institutes. □

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Ring the changes

Stanley Roberts

Polycyclic Aromatic Compounds. Editor-in-chief M. Zander. *Gordon and Breach*. 4/yr. UK Dfl. 1287, rest of Europe ECU 557, US and Canada \$595, elsewhere SwFr. 1170 (corporate); Europe Dfl. 825, US and Canada \$375, elsewhere SwFr. 750 (academic library); UK £108, rest of Europe ECU 179, US and Canada \$168, elsewhere SwFr. 384 (personal).

THE aim of this new journal is to collate information from scientists working with polycyclic aromatic compounds in biology, physics and chemistry laboratories. Short papers, full-length articles and reviews are welcomed.

Polycyclic aromatic compounds are undoubtedly important — for example some products of their oxidation are carcinogenic. Nonetheless, it is doubtful whether they justify a journal all to themselves, especially considering the current huge proliferation of journals.

(Although admittedly, sophisticated computer-aided searches can now help scientists sift through the massive volume of published literature.)

The times between submission and publication for the articles in the 1990 issues will not be attractive to potential contributors, being long (10–12 months) compared with those of leading international journals, such as *Perkin Transactions* (4–6 months); but I guess this situation will improve if the journal becomes more popular, and as the refereeing, editing and printing of the papers becomes commonplace.

The journal will be a useful vehicle for scientists working with polycyclic aromatic hydrocarbons. It would also be of interest to chemists and biologists working in fields that are often relevant to these compounds, although I doubt whether many of these researchers will get around to reading it. □

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Great shakes

Derek Steele

Vibrational Spectroscopy: A Section of *Analytica Chimica Acta*. Editors J. G. Grasselli and J. H. van der Maas. *Elsevier*. 4/yr. Dfl. 332.

THE declared aim of this new journal is to "assemble the research findings in the field of vibrational spectroscopy, now scattered through the literature, in a single journal." There are, however, several journals already in existence devoted to this subject (*Spectrochimica Acta*, *Journal of Molecular Structure*, *Journal of Molecular Spectroscopy* and so on). So it is difficult to see how the publication of this new journal can be justified. Nevertheless, journals continue to proliferate despite library-funding crises. Accepting this inevitability although not its logic, what has *Vibrational Spectroscopy* got in its favour?

The editors are spectroscopists of high calibre and can be relied upon to maintain a high standard of refereeing and editing. Indeed, several well-written articles in the initial issues describe important new developments. Most papers are on analytical aspects of spectroscopy, as is proper for a journal which is an off-shoot of *Analytica Chimica Acta*. Also, there are some good theoretical papers — witness the article by Gantz *et al.* entitled 'Continuum resonance Raman scattering in diatomic molecules'.

Articles on Raman spectroscopy appear as frequently as those on infrared spectroscopy, and there are many papers

on technique developments. Depth-profiling, Langmuir–Blodgett films, photoacoustic techniques and microprobe investigations of electrodes are all covered in the first four issues.

In addition to these research publications, some excellent review articles are included, such as H. J. Luinge's review in the first issue of automated interpretation of vibrational spectra.

Half of the second and all of the third issues consist of selected papers that have arisen from conferences. With so many journals in competition for good material it is inevitable that this means of acquiring copy should be prevalent. It is, however, somewhat undesirable in that conference proceedings more often than not consist of material that is being published elsewhere. Having said that, I confess that I found such papers presented here interesting and of good quality.

The journal has got off to a good start and I wish it well. It would have been nice, however, if its birth was accompanied by the demise of a journal elsewhere. Unfortunately, such idealism does not seem to have a place in this practical world. □

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Seamless joins

Michael J. Gait

Bioconjugate Chemistry. Editor-in-chief Claude F. Meares. *American Chemical Society*. 6/yr. US \$249, Canada and Mexico \$257, Europe \$266, elsewhere \$273 (nonmembers); US \$29, Canada \$37, elsewhere \$46 (members).

"*BIOCONJUGATE Chemistry* addresses the joining of two different molecular functions by chemical or biological means". This quote from the journal's advertising blurb prompts the immediate reaction: "Why do we need a journal for this?"

The answer is quickly found by browsing through a few recent issues. First, many different chemical and biological subjects are represented; before this journal came into being in 1990, the topics represented were scattered throughout many disparate journals. More importantly, the journal has an immediate and wide-ranging appeal. This is partly due to the basic fascination of observing what happens when two dissimilar molecules are joined together: the result is not always the sum of the parts. But the unifying theme is hinted at by the prefix 'Bio'. Beyond the mere

joining of two molecular species, there is a clear and overriding aim to generate novel and useful combinations of biological and chemical properties within a single entity. This sense of purpose nicely ties in the various disciplines and provides a tangible justification for the journal's existence.

Some topics are more highly represented than others, notably immunoconjugates. But there is a healthy spread of papers involving proteins, polypeptides, oligonucleotides, oligosaccharides, drugs and other biomolecules. The juxtaposition of these topics is enjoyable, because one is exposed to a wide variety of reagents and techniques from different fields. So the journal provides a focus for the interchange of ideas and techniques between disciplines.

For a fledgling journal, there are many solid and reassuring aspects of style. Both text and figures are easy to read and occasional issues carry excellent full-colour reproductions. Experimental procedures are rightly featured before the results and discussion sections, and there is the usual high standard of chemical characterization expected of a journal from the American Chemical Society.

Apart from articles and reviews, there are letters (essentially short articles of less than 1,000 words) and, unusually, an occasional teaching editorial, which will be helpful to the many nonspecialists who are likely to read the journal. There is no doubt that it will occupy an important niche on library and personal bookshelves for many years to come. □

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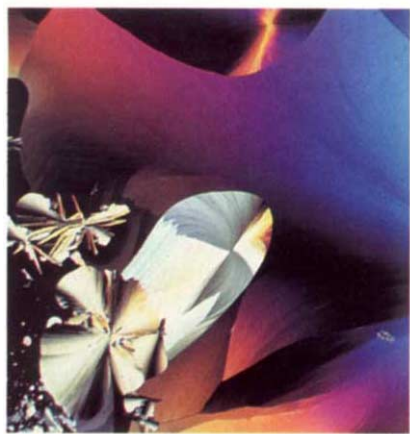
Fostering interactions

Michael G. B. Drew

Structural Chemistry. Editors István Hargittai and Arthur Greenberg. *VCH*. 6/yr. \$295, £195, DM 607 (institutional); \$95, £78, DM 210 (personal).

THE aim of this journal is to encourage a broad spectrum of articles under the general title of structural chemistry and hence to foster interaction between branches of structural chemistry. Structural chemistry is defined to include experimental structure determination, experimental thermochemistry, and other relevant techniques such as photoelectron spectroscopy, as well as computational studies, new structural models and bonding theories.

These aims have been well achieved in



Polarized light micrograph of crystals of phloroglucin ($\times 14$).

the articles published in 1990, which are of consistently good quality. Most of the papers combine experimental and theoretical studies and cover a wide range of organic and inorganic systems. Popular topics include molecular mechanics and quantum mechanics calculations as well as single-crystal and electron-diffraction structure determinations. The articles are well presented, and the journal is a pleasure to read. It looks like becoming a high-quality journal in an important and expanding field.

But what about the competition? There are already several journals that cater for the structural chemist but these are mainly restricted to one topic or another. Few encourage the breadth of topics that are covered in *Structural Chemistry*, where the juxtaposition of articles about different areas is a particularly pleasing aspect.

Recent advances in computer technology and development of theoretical techniques now enable us to investigate interesting molecules of a fairly large size. Results from these studies need to be compared with experimental work, and for that reason alone, this new journal is to be welcomed.

Is the journal necessary? Certainly the articles here could be usefully published in several other journals. But as published scientific material expands every year, new journals are necessary and this one has found a suitable gap in the market-place.

The stated aim of *Structural Chemistry* is to overcome the unnatural separation that exists in the current literature among the sciences of structure determination, energetics and applications, and to build a bridge to other chemical disciplines.

The journal has made a considerable impression in its first year of publication and I recommend it to all structural chemists. □

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Tying together

R. A. Pethrick

Polymers for Advanced Technologies. Editor-in-chief Menachem Lewin. Wiley. 8/yr. \$450 (institutional); \$340, £185 (personal).

POLYMERIC materials are finding increasing use in the development of new materials for advanced technologies. This journal attempts to provide a focus for research in this area, and deals with organic, inorganic, multiphase and composite polymers with application in electronics, information science, aerospace-related industries, automotive industries, packaging, imaging, integrated circuit manufacture, agriculture and pharmaceutical industries. The topics covered are divided into four subheadings: radiation-related polymers, advanced structures, polymers for biosystems and materials. The journal provides a suitable focus for papers that are currently finding an uneasy home in more conventional single-discipline journals, and addresses the multidisciplinary nature of much of the work in this area. The first volume covers topics spanning poly-

electrolytes, liquid-crystalline polymers, electro-optic properties, photocrosslinking systems, resists and piezoelectricity.

The A4 format of the journal is clear and easy to read, but the reason for the second issue being smaller in size is not clear. The inclusion of a key-word index is valuable considering that many of the papers are of a multidisciplinary nature. One of the papers in the first volume contained eight pages of theoretical calculations on heat capacities, information which was also presented in graphical form and in terms of the component equations. Duplication of information of this sort is rather expensive and should be avoided; perhaps the policy adopted by other journals of providing supplementary information on request would be a useful ploy here, and would reduce such large amounts of tabular material. The reproduction of electron micrographs in two of the papers was very poor, causing information to be lost for the reader.

Despite these criticisms the journal makes a good overall impression and is likely to fill a gap for those involved in the development of new materials for advanced technologies. □

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Solid start

Paul Calvert

Journal of Materials Chemistry. Editor A. R. West. Royal Society of Chemistry. 12/yr. European Communities £280, US \$599, Canada \$331, elsewhere £315 (institutional).

OVER the past few years materials have become recognized as a key technology for industrial growth. This has been expressed by the growing funding for materials science with promises, in the United States, of larger increases to come over the next year or so. The result of this public attention has also been that solid-state physicists, and inorganic and solid-state chemists, have realized that they are really materials scientists too. The true materials community is becoming nervous about being crushed in the stampede by these large herds. Both the American Chemical Society and the Royal Society of Chemistry have produced journals to reflect this materials involvement among chemists. The American Chemical Society journal *Chemistry of Materials* is now in its third year. *Journal of Materials Chemistry* was started this year by the Royal Society of Chemistry.

This participation by chemists also reflects a shift in emphasis in materials

science. The traditional heartland of materials science is the exploration of the effect of changes in microstructure on properties. Thus strength in metals or ceramics is affected by annealing treatments or alloying additions, which modify grain size. The starting metal, ceramic powder or polymer, was usually supplied by industry.

Nowadays there is far more emphasis on new materials such as the high-temperature superconductors, nonlinear optical polymers and precursor polymers for ceramics. Physicists are needed to predict what molecular structures will have the desired properties and chemists are needed to make these structures. The real materials science starts when we have identified a high-temperature superconductor and want to turn it into a wire with usable strength and current-carrying capacity.

These two new 'chemistry and materials' journals serve this field of synthesis of solids, as opposed to the more usual synthetic goal of making soluble compounds with new types of bonding. In his introductory editorial to *Journal of Materials Chemistry*, Anthony West describes its area as synthesis or fabrication, structure determination and property measurement of both organic and inorganic materials. Many of the materials papers from chemistry departments do emphasize synthesis coupled

with spectroscopic characterization and have not looked comfortable in the pure materials journals such as *Journal of Materials Science*.

As an added encouragement *Journal of Materials Chemistry* has no page charges (neither does *Chemistry of Materials*) and offers 50 free reprints.

The huge development during the 1970s of new materials for integrated circuit manufacture was mostly concentrated in electrical-engineering departments, while the materials scientists were still worrying about bending paper clips. It will be interesting to see if we miss this next revolution too. □

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Repeated pleasures

N. McN Alford

Journal of Materials Science: Materials in Electronics. Editor Arthur Willoughby. Chapman and Hall. 4/yr. EC £95, US and Canada \$170, elsewhere £103.

THIS journal has an excellent pedigree. It forms part of the *Journal of Materials Science*, which has a wide readership, is well presented and is essential reading for anyone with an interest in materials. The new journal is more focused but retains the same format and reproduction quality as its parent. It is published quarterly and is free if you already receive *Journal of Materials Science*. I hesitate to suggest to anyone that they should pick up yet another journal, but this one fills a specialist niche — that of the use of materials in electronics — and for this reason it is likely to be successful. In fact, this means that its territory is the whole range of materials from metals through ceramics to polymers, and the issues I looked at covered a wide range of their electrical and electronic properties. These included ionic and electrical conductivities, dielectric, thermoelectric, photoconductive and ferroelectric properties, as well as properties of superconducting materials. Preparative techniques and properties of the materials were discussed with special emphasis on the electronics. The editorial in the first issue emphasized the importance of applications-based research. And sure enough, I found much science that will be useful in applications, although only one or two of these were mentioned specifically.

The most serious competition to the new publication will come from *Journal of Applied Physics* and *Applied Physics*

Letters, both of which discuss the materials aspects of electronic materials. Some journals, such as *Journal of Physics and Condensed Matter* and *Electronics Letters*, generally assume that materials science is a necessary chore but not really worth examining in detail. In a sense this is understandable — these are journals about physics or electronics. But when things begin to go wrong it is usually a materials problem. A shining example of this can be found with high-temperature superconductors. Here the processing of the materials, the choice of substrates, the orientation of the superconductor and the substrates, the introduction of weak links and the elimination of weak links, are all materials problems. To be fair, most 'pure' physicists or chemists are not that pure. They have all had to tackle such problems from time to time, which indeed always require a multidisciplinary approach.

The content range of the new journal is excellent, and to the researcher interested in electronics, the juxtaposition of papers on such a broad spread of materials will be fascinating. The only reservation I had was that in some cases I had read very similar material by the

same authors in other journals. The solution is tighter refereeing.

The one review article I saw was on radiation damage in solar cells by T. Markvart. This was very good and one hopes that such quality reviews can be maintained. In one or two other articles I was struck with the thought: why have the authors done this and is it useful? For example, much is known about plasma spraying and there have been several papers on the application of this technique to oxides. The paper by Hibino *et al.* on plasma spraying of oxide superconductors in the first issue was therefore not particularly novel. So why include it? More importantly, what are the electronic properties of the product?

The addition of yet another journal requires serious soul-searching. In this case those who get to see the new arrival as part of its parent journal and who are interested in electronics will be very pleased. For others, it is well worth reading a few copies and then deciding whether to subscribe. □

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Mature matters

John Nicholson

Journal of Materials Science: Materials in Medicine. Editors W. Bonfield and D.F. Williams. Chapman and Hall. 6/yr. EC £135, US and Canada \$240, elsewhere £145 (institutional); members' rates available on application to publisher.

IN recent years the study of materials for use in medicine and dentistry has developed dramatically. Just over 30 years ago, J. Charnley, the orthopaedic surgeon, devised substantial improvements in hip-replacement surgery yet used denture-grade poly(methyl-methacrylate) to secure his new prostheses in place. There was little understanding of the mechanical or biological properties needed. All that has changed now. The interdisciplinary science of medical materials has come of age.

To mark this maturity, the new publication *Materials in Medicine* has been launched, the first edition appearing in June 1990. It is published in association with the well-established *Journal of Materials Science* and aims to cover the science of the metals, ceramics, polymers and composites used in a range of medical areas, including orthopaedics, maxillofacial and cardiovascular surgery and dentistry. Publication started as quarterly, but is now every two months.

The first editions more than fulfil the

publisher's aims, and cover work ranging from the porosity of zinc polycarboxylate dental cements to surface modification of medical grade PVC, and from preparation of synthetic hydroxyapatite to enzymic hydrolysis of biopolymers. The quality of these papers is high and the authors can have no grumbles about the time between acceptance and publication, which in most cases was under six months. I will watch with interest to see if this speed can be maintained in the future.

So far, so good. But I must sound one note of discord. There are now many journals operating in this field (*Clinical Materials*, *Biomaterials*, *Journal of Biomaterials Science*, all from the United Kingdom; plus the *Journal of Biomaterials Science* from the United States) together with a number of specialized journals on the narrower subject of dental materials.

Having refereed papers for several of these journals, I find it hard to believe that there are sufficient people doing work in biomaterials of the necessary quality to sustain all these publications. Consequently, some of these titles may well not survive. If that is so, I hope *Materials in Medicine* is one that does continue. Given the quality of the first few editions, I think the editors, Bill Bonfield and David Williams, deserve to succeed. □

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No slip-up

L. Crombie

The Journal of Essential Oil Research.

Editor-in-chief Brian M. Lawrence, Allured Publishing Corporation, 214 West Willow, PO Box 318, Wheaton, Illinois 60189, USA. 6/yr. US and Canada \$150, elsewhere \$170.

THE term 'essential oil' does not convey anything very precise to many people. Indeed, it is a broad term, signifying the mixture of volatile organic compounds that produces the essential odour or taste of the vegetable substance from

*Levande minor, fusc Spica,
Lavender Spike.*



which it is derived. Evocative names like rose, eucalyptus, lavender, lemongrass, peppermint, patchouli, bergamot, vetiver and ylang-ylang are typical. Generally the compounds are produced by steam distillation or solvent extraction of suitable plant material, although occasionally more subtle and expensive means are employed to retain a delicate fragrance, as in the enfleurage process for jasmine products. Here the odorous compounds are collected by absorption into odourless lard from batches of flowers spread repeatedly onto glass plates smeared with the lard.

Commercially, essential oils are of particular importance in the perfumery and food-flavour industries; some are bulk commodities, some are extremely rare and expensive. Coming as they do from all parts of the globe, their supply depends on climatic and political circumstances, and, especially in the case of rare oils, price and supply can fluctuate and lead to adulteration with cheaper materials. Consequently, this area of analytical science of essential oils is an important, if specialized, one.

Organic chemists, too, have always been fascinated by essential oils; indeed, they have played an important role in the development of chemistry. The great German chemist Otto Wallach undertook prolonged investigation of essential oils from around 1880 onwards. This was a time when scientific knowledge and

techniques were ill suited to dealing with such complex, often liquid, mixtures, yet by his labours he laid the foundation of terpene chemistry as we know it. Today life is much easier for the essential-oil chemist. Mass spectrometry coupled to separating equipment such as a gas-liquid chromatograph, a high-performance liquid chromatograph, or a second mass spectrometer, makes short work of the extremely complex mixtures if the components are already known and represented in the mass-spectrometry file. Even if new, much progress can be made on such structures. With powerful qualitative and quantitative tools such as these, adulteration is comparatively easy to detect.

The aims of the new journal, however, are much wider than this. It sets out to be

devoted to all aspects of pure and applied essential-oil studies as summarized in the following categories: agriculture and horticulture, analytical chemistry, biological activity, biotechnology, chemical composition, chemical synthesis, chemosystematics, microbiology, plant biochemistry and biosynthesis and toxicology. Time will tell whether all these areas will be strongly represented in the journal. It accepts reviews, research communications, research reports, research letters and research notes, all in English. The journal is well produced on high quality paper, but personally I would have liked a larger format. □

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Nouvelle cuisine

Gordon C. Cheeseman

Trends in Food Science and Technology.

Editor John O'Brien. Elsevier. 12/yr. £239 (institutional); UK £59, US and Canada \$92, elsewhere £63 (personal).

THIS new journal in the *Trends* series was launched by Elsevier in July 1990. A principal objective of the journal is to monitor and assimilate the significant innovations from a wide range of research areas, in order to stimulate research in the food sector.

Food science and technology is multidisciplinary, and a new journal in the field will always have the problem of maintaining the interest of specialists working in many fields. These fields include, for example, engineering, physics, chemistry, microbiology, nutrition and also involve the vertical spectrum of interests from basic food production, through processing, to diet and health.

The format used is of the magazine style and is ambitious in the number of sections proposed. These comprise features, reviews, viewpoints, letters, conference reports, book reviews, software reviews, technical advice and details of forthcoming events. But not all of these sections are included in each issue.

The main part of the journal contains review articles. The policy of keeping the reviews short and readable together with that of having a mix of topics in each issue is particularly valuable, as nonspecialists will be interested and kept informed. Specialists will find more extensive reviews published elsewhere; however, one advantage of a journal of this type is that the short publication time enables up-to-date research to be covered. The reviews appearing during

the first year of publication have, in the main, been well written and usually sufficiently critical to enable the reader to obtain an appreciation of the likely impact and progress of the area of science covered. This aspect is important as the lag period between scientific advances and practical applications can be extensive if the important implications are not widely understood within the industry. There are usually three reviews per issue, and topics covered during the past year have included the detection of irradiated foodstuffs, molecular dynamics of food proteins, and electrical detection of food-borne microorganisms.

Interesting and useful inclusions are the viewpoints — occasional articles by leading experts in which more contentious areas are discussed. These have included articles on toxicity of disease-resistant plants and water activity — a credible measure of food safety and quality. Such articles succinctly draw attention to specific problem areas for the food scientist, giving useful criticism of the body of published papers and highlighting problems with existing concepts.

The conference reports, instrumental and technical advice applicable to food research, book reviews and letters, all contribute to the magazine style of the journal.

Increased public awareness of diet and health relationships, changes in both life styles and retailing and manufacturing of food, has meant that a great deal of attention is now given to food research both in the public and private sectors. Interactive journals, such as *Trends in Food Science and Technology*, will play an important part in supporting the diverse and rapidly advancing area of food science. □

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Sizing it up

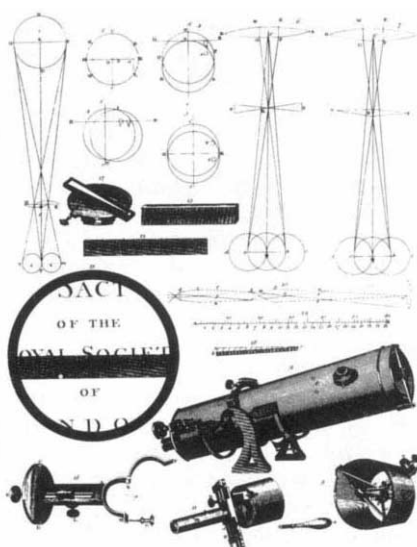
John Gallop

Measurement Science and Technology.
Honorary editor P. A. Payne. *IOP*. 12/yr.
UK £245, elsewhere \$490.

NOWADAYS only a few new journals buck the general trend towards greater and greater specialization. That *Measurement Science and Technology* is one of these is less surprising when one appreciates that it is not an entirely new journal but something of an Institute of Physics reincarnation, having begun life under the title of *Journal of Scientific Instrument* and appearing more recently as *Journal of Physics E*.

The editors solicit papers or short notes describing new measurement methods, devices or instrumentation across the whole range of physics, chemistry and life sciences. In addition to research papers, a regular section of shorter design notes is included in every issue, as well as occasional review papers on topics of wider interest (for example, optical aperture synthesis), conference reports, book reviews and a short essay section, in which individuals can air their views on topics having a possibly tenuous relationship with measurement science. I suspect that the aim of the editors is to broaden the scope of *Measurement Science and Technology* so that it approaches, admittedly only within an instrumentation horizon, the treatment by prestigious multidisciplinary research and news journals such as *Nature* and *Science*.

The new sections form a relatively small part of the journal. Former readers of *Journal of Physics E* will still find much that is familiar: stacks of papers describing novel forms of transducers to measure displacement, temperature, magnetic fields and fluid velocity, but many others which are frankly esoteric. Thus the paper on page 1 of volume 1 in January 1990 was entitled 'An accelerated life-test for bicycle freewheels' and dealt with a test rig for evaluating bicycle components under realistic conditions. Papers tend to be more practical than theoretical. This is a journal that tells its readers how to do experiments, rather than proposing what might be done or even reporting the results of what has been done. From the issues published so far I would estimate that the number of contributions from chemistry and the life sciences are running a very poor second and third to physics. This is scarcely surprising in view of the publishers and the readership of the precursor journals, but it will be interesting to see if the editorial board can translate desire for general coverage into reality.



Old devices — micrometers used in 1810.

Already the range of topics covered is great and the originality of many contributions high. I defy any experimental physicist to look through an issue with-

out being surprised by something of unexpected relevance or interest to their own work.

Browsing through an issue is perhaps the only way to allow such interdisciplinary seeds to take root. No combination of keywords and on-line databases seems able to produce such cross-fertilization. On the other hand, do today's overworked scientists have time to browse through monthly journals of this size in the hope of coming by chance on a paper that can provide the crucial piece of information they have been needing for months? Surely this is the dilemma that the editors hope to resolve by adding editorial and review material; browsing then allows the acquisition of both broader and narrower knowledge, so that the time allotted to reading the journal will therefore have been more effectively spent. □

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Knowledge bank

B. Wu

Journal of Intelligent Manufacturing.
Editor-in-chief Andrew Kusiak. *Chapman and Hall*. 6/yr. Europe £120, US and Canada \$215, elsewhere £130 (institutional); Europe £45, US and Canada \$80, elsewhere £45 (personal).

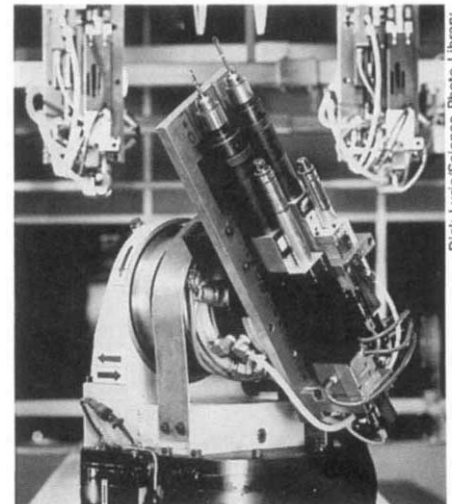
ONE cannot overstate the importance of manufacturing in an industrialized country, for it is mainly through this activity that the nation's real wealth is created. Today, everyone either is directly involved with manufacturing industry or benefits from its products. Therefore it is vital continuously to improve manufacturing performance. Both the means of manufacturing and the structures of manufacturing systems, as well as the environment within which they operate, have recently radically changed.

So far there have been three stages of industrial development: the neolithic revolution (the age of craftsmanship); the industrial revolution (the age of mechanization); and the new industrial revolution (the age of information and automation). The manufacturing industry is now in the midst of the third of these, the technological revolution, which is characterized by the increasing use of computers for both information processing and automatic control. Whereas the first two stages of industrial development related to hardware (tools and machines) to increase the physical abilities of man, the latest revolution in manufacturing puts more emphasis on information and control to heighten his mental capabili-

ties. This shift is reflected by the publication of this new journal.

The term "intelligent manufacturing" refers to the use of artificial intelligence to improve the effectiveness of manufacturing, including, for example, product design, process design, systems design, strategy development and management operations. Many papers on the subject have already appeared in several journals related to advanced manufacturing technology. As the number of research projects and practical applications increases, the size of published literature in the field has grown quickly. With well-focused and defined objectives, this new journal provides a welcome, encompassing source of much needed information for both academics and professionals involved in intelligent manufacturing.

The journal puts much emphasis on the value of real applications — all of



Arm of an industrial robot that drills and deburrs holes.

the papers are application-orientated. One cannot fail to be impressed by the diversity of topics covered. This clearly reflects the wide applicability of artificial intelligence to manufacturing. Indeed, one of the most desirable features of the journal is that it provides a rare panoramic view of this subject which is otherwise quite difficult to obtain. On the whole, the publication is well balanced between papers offering solutions to particular problems and those addressing more general issues. There are also special issues that are devoted to particular topics such as neural networks in manufacturing, manufacturing and decision processes and intelligent heuristics for the design of manufacturing systems. It may be argued that a few of the papers lack a level of academic abstraction — they seem to be more like extracts from a textbook rather than academic papers seeking to expand the frontier of know-

ledge.

Nevertheless, they provide interesting and useful accounts (such as that of the design approach of fuzzy control processes), and the choice of topics that they cover appears to be in line with the editor's objective of seeking to bridge the theory-practice gap.

In this era of rapid technological changes, I am sure that those involved in the research, development and application of advanced manufacturing technologies, particularly those who are interested in the applications of artificial intelligence in manufacturing, will join me in congratulating the editors and publisher for producing a timely, good-quality journal that successfully bridges the gap. □

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Net profit

Igor Aleksander

International Journal of Neural Systems. Editor-in-chief Benny Lautrup. *World Scientific*. 4/yr. \$190, developing countries \$65 (institutional); \$86 (personal)

IF the publication of John Hopfield's 1982 paper on neural networks (*Proceedings of the National Academy of Sciences U.S.A.* 79, 2254–2558) can be blamed for the resurgence of interest in this field, it is interesting to note that 1991 marks the last year of a decade of this revival. Have great things happened? Is there promise still ahead? These are the questions that will determine the life span of the journals that are steadily proliferating in this field. *International Journal of Neural Systems* must be among 15 or so similar projects, all of which are competing for the attention of about 8,000 neural-network researchers in the world. While publishers are quick at seizing the opportunities offered by this sort of bandwagon, the acid test is whether the quality of papers in the journal is sufficiently high for researchers to add it to their reading list.

On the strength of the first volume, I think that a reasonable quality has been achieved. The range is broad, covering mathematical, biological and engineering interests. Duplication with the content of other journals is low and editorial filtering (or just fortune?) has kept the quality of the papers high. An interesting aspect is the distinct European flavour of the papers, and this is not a bad thing. While there are contributions from California and Australia, the peak of the geographical distribution of contributions is somewhere in the middle of the

Alps, with the standard deviation ranging between Scandinavian countries and Israel. Without wishing to upset my colleagues in the United States, it is palpably true that 'European' in neural-net research implies a greater variety of ideas than can be found purely on the American continent. This may be because the United States is the land where, in 1969, Marvin Minsky and Seymour Papert ruled that "thou shall not work on neural networks". The post-1982 US researchers then found themselves picking up the trails of a truncated scientific culture where Europeans have had the benefit of the maturity that comes from unbroken experience. So they look for new pastures with greater confidence. There is evidence of this in this new journal.

Everyone has a list of important developments they would like to see in neural networks. Mine contains systems with many associative nets, hybrid systems that integrate conventional and neural computation, neural nets that understand language aided by images, and nets that are truly sensitive to complex temporal structures. Perhaps it would be unreasonable to expect that such material would be found in the first volume. Some is, and the key thing for the survival of a journal such as this is whether it can encourage and attract advanced contributions on new developments. It will be up to the 41 associate editors to be clear about their own wish lists, and for them to ensure that what is published is truly new and not merely the twist of a synapse in an ancient neural model. □

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Binary bibles

John A. Campbell

International Journal of Foundations of Computer Science. Editors E. Engeler, T. Ito, D. J. Lee, R. Parikh and J. V. Tucker. *World Scientific*. 4/yr. \$195 (institutional); \$82 (personal, and institutional for developing countries).

Mathematical Structures in Computer Science. Editor G. Longo. *Cambridge University Press*. 3/yr. \$135, £75.

Computational Mathematics and Modeling. Editors W. A. Light and A. N. Tikhonov. *Plenum*. 4/yr. US and Canada \$295, elsewhere \$345.

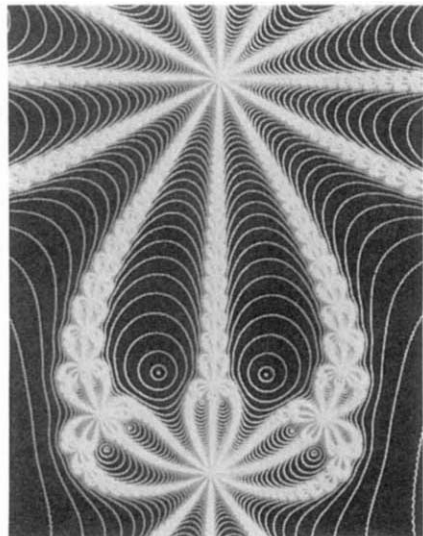
Journal of Visual Languages and Computing. Editors S. K. Chang and S. Levialdi. *Academic*. 4/yr. US and Canada \$140, UK £72 (institutional); US and Canada \$60, UK £72 (personal).

COMPUTING is a popular field for new journals, many of which are responses to the increased amount of important work in established topics. Others recognize or attempt to define new topics. There are examples of both in the selection reviewed here.

Theoretical computer science and the foundations of computing are certainly undergoing an expansion of activity at present. Although some of it is due to displaced mathematicians looking for a larger and possibly richer audience, there is enough fire to justify the amount of smoke that is visible. *International Journal of Foundations of Computer Science* takes its share of the action by accepting papers in quite a broad theoretical spectrum, with emphasis so far on algorithms and their properties, programming-language semantics, and aspects of logic programming. It does not lack competitors, such as *Theoretical Computer Science*, *SIAM Journal of Computing*, *Journal of Algorithms* and *Acta Informatica*, and evidently supplements them rather than offering a distinctive new profile. For those who have the money, it is a useful supplement.

Mathematical Structures in Computer Science is more specialized. It relies on the correct assumption that progressively more mathematical territory (mainly algebraic, not the typical analytic kind of applied mathematics used in other sciences) is being colonized, or at least raided for items of value, by computer scientists. Some of its contents are uncontroversial, in the sense that they could have appeared in any of the above journals. The remaining papers are less obviously items that raiders from computer science will want to capture, but the journal disarmingly admits this in print

by hedging its bets about the attractiveness of its favourite subjects, category theory in particular. The choice of emphasis is therefore an act of faith that the mathematical flavour, if a little astringent for computer scientists in 1991, will be routine within a few years. Theoreticians may wish to reward this



Gregory Sams/Science Photo Library

Fractals — patterns of popularity.

high-risk approach with a subscription now; others can probably afford to wait.

Computational Mathematics and Modeling is a journal founded to publish translations of Soviet papers, mostly from the *Transactions of the Faculty of Computational Mathematics and Cybernetics* of Moscow State University. Section headings include numerical methods, methods for solving inverse problems, and mathematical modelling. The style is classical: for example, "modelling" has the meaning accepted in applied mathematics for many years, and there is no trace of more recent Western preoccupations such as ecology, dynamical systems, or even computer configurations and networks. But given the quality of Soviet computational mathematics and the increasing communication with contemporary computer science outside the Soviet Union, this is almost guaranteed to change, although the results may not be evident in the journal for some time.

Journal of Visual Languages and Computing is the only journal in the set that tries to establish a standard for a new field. Apart from what the name suggests, the contents cover pictorial information systems and databases, use of icons in programming, biologically inspired vision systems, visualization of data, and associated issues of user modelling and the human-computer interface. As many papers on these subjects are now turning up in conference proceedings and a variety of journals, there is a good case for having a single recognizable focus for all of this effort. The

papers in the first volume are consistently enlightening about what can or ought to be included in visual computing, and do full justice to its rather heterogeneous mixture of components. In addition, the editors have allowed into their pages a quotation from the software engineering guru F. Brooks that expresses scepticism about their subject. This combination of openness and adventurousness in the journal deserves a positive response. Unlike the other journals under review, this publication takes on something new and has no real rival. □

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Seeing the light

W. J. Firth

Quantum Optics. Editor E. R. Pike. *IOP* 6/yr. UK £139, elsewhere \$278.

QUANTUM theory famously sprang from the inability of classical radiation theory to predict the black-body spectrum of thermal radiation, by way of Planck's 1900 hypothesis that elementary radiation sources can exchange energy only in discrete multiples of their oscillation frequency. One might therefore expect the 'photon' or quantum of optical energy to be the keystone of twentieth-century optics.

Why, then, should it have taken almost a century for it to have a journal of its own? Basically, Planck's quantum of light was upstaged almost from square one by Bohr's extension of the quantum hypothesis to atoms, which led on to all of atomic, nuclear and solid-state physics, much of modern chemistry and much else besides. Not only was the photon neglected, it was even reviled as unnecessary: almost all radiation phenomena, including thermal radiation, can be explained satisfactorily by coupling quantized matter to classical radiation — the so-called semiclassical model.

Only in the past few decades has it become possible to devise and perform experiments in which the quantized nature of light is essential, and in which there is a measurable difference between quantum and classical theories. In every case decided so far, the quantum nature of light has been vindicated, and these experiments, together with the development of the associated ideas and their consequences, constitute the new science of quantum optics — the study of phenomena that require nonclassical theories of light for their understanding.

Most of the earlier phenomena were rooted in the statistics of detectors, for

example photon antibunching. Today's hottest topic is squeezing, that is, getting around the inherent graininess of quantum light and apparently beating Heisenberg's uncertainty principle by taking advantage of the fact that it is the product of quantities that has a minimum uncertainty: one factor can have a very small error provided that the other becomes very uncertain. Schemes for ultra-high-precision measurements, and possibilities for low-noise communications, give squeezing both a fundamental and an applied dimension — a powerful combination in these times.

This journal aims to be by and for the 'strictly quantum' optics community. This is a fairly small and widely scattered community, and the journal has been slim (a total of 30 contributions in 1990), if geographically broad in its author catchment. It can hardly be said to have superseded existing journals for which quantum optics is a subtopic. For example, *Physical Review A* continues to publish many more articles on quantum optics than *Quantum Optics*.

The plot thickens, however. Early this year the European Optical Society was formed, with one of its aims being to emulate and rival the Optical Society of America, whose journals dominate the wider domain of optics, with strong competition, especially on the European front, from *Optics Communications* and *Journal of Modern Optics*. Among its activities, the society will publish a two-part journal, one part being devoted to "pure and applied optics", with *Quantum Optics* as the second part.

Cultivating a quiet if fertile corner of optics is one thing: taking on the entire field is quite another. The future of this infant journal looks interesting but somewhat uncertain. □

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Treading over old ground

C. J. S. Clarke

Reviews in Mathematical Physics. Editor-in-chief Huzihiro Araki. *World Scientific*. 4/yr. US \$265 (institutional); \$106 (personal).

THE title of this new journal is perhaps unfortunate, raising expectations that are impossible to fulfil in publishing terms. The ideal review would be a pedagogic work, carefully aimed at an audience of workers in different fields who either are entering the field reviewed or require background informa-

tion about it for their own work. It would cover the entire area with a noble disregard for the author's own predilections, and provide the definitive bibliography for all subsequent research students. In practice, this journal, like many others similarly named, embraces a range of styles from the unashamed research article, through expositions of a new approach that is very pretty but has not produced any results yet, to articles (such as that by Stephen Summers) on the independence of local algebras in quantum field theory that almost reach the ideal. And what of 'mathematical physics', a term whose meaning narrows with every passing year? The scope of the journal turns out, with few exceptions, to be the most mathematical (and on the whole algebraic) end of quantum field theory and of soliton theory, verging on, nay often massively invading, pure mathematics. The scope is thus a proper subset of that of *Communications in Mathematical Physics*. The issues I inspected did not, for instance, cover general relativity (except as a source of

interesting infinite dimensional Lie groups) or classical applied mathematics. Applied physicists would be forgiven if they assumed that the authors had no interest in 'real physical problems', even though this is very far from the truth.

The articles, although all interesting and of good quality from reputable authors, often had a tendency to be lightweight, as if the editors were still soliciting articles from prestigious friends who did not actually have anything new to say. Unfortunately, several articles were marred by constant grammatical errors that the editorial staff had failed to correct. Despite this, the impression is of a well produced journal bringing in good material, but in the end failing to achieve anything that is not already covered by the journals *Physics Reports* and *Communications in Mathematical Physics*. □

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Star wars

A.W. Wolfendale

Experimental Astronomy. Editors Jacques M. Beckers, Webster C. Cash, Thijs de Graauw, Robert J. Hanisch, Stephen S. Holt, Richard T. Schilizzi, Volker Schönfelder. *Kluwer*. 4/yr. Dfl. 240, £81.50, \$122 (institutional); Dfl.140, £47.50, \$71.50 (personal).

THE prospect of yet another international journal fills most scientists, and librarians, with horror, so I approach my task here with considerable scepticism and the need to be convinced of the new journal's value.

Experimental Astronomy is an "international journal on astronomical instrumentation and data analysis" launched in 1989. At first sight the papers represent a rather miscellaneous collection, with topics drawn from a variety of areas, from TeV gamma rays to rocket-borne far-ultraviolet spectrographs. On reflection this is inevitable in a journal devoted essentially to techniques.

The papers read as something intermediate between the 'normal' scientific contribution and a PhD thesis, so only occasionally will readers find something of considerable interest. Libraries of adequate means should be encouraged to subscribe to the journal, but I doubt whether there will be many personal subscribers. □

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Eco-ethology?

Darryl T. Gwynne

Behavioral Ecology. Editors Staffan Ulfstrand and Donald L. Kramer. *Oxford University Press*. 4/yr. US and Canada \$130, elsewhere \$140 (institutional); \$39.50 (members); \$22.50 (student members).

ABOUT 16 years ago a reviewer of my thesis proposal commented on my use of 'behavioural' by asserting that it did not exist in the *Oxford Dictionary*. Today certain biologists feel such an adjective to be sufficiently appropriate to describe their approach to ecology that it has been used as the title of at least four books and, with the addition of this new periodical, two journals.

Behavioural ecology usually refers to the study of behavioural (and related morphological) adaptations of organisms to their physical, biotic and social environments. Practitioners of this study area convened at the first meeting of the International Society of Behavioural Ecologists in 1986 and, at their second meeting in 1988, enthusiastically supported a new journal. Thus an important service of *Behavioral Ecology* is to the society, publishing not only original papers but also its editorial policy and announcements. But a good journal should be more than a 'societal organ'; more importantly, it should fill a need. Does it in fact succeed in doing this, especially given the existence of the similarly titled *Behavioral Ecology and Sociobiology*? The answer seems to be

yes for two reasons. First, there is room for two journals in the subject area because of the rapid growth in behavioural ecology research in the past 10 years or so. This is evidenced by the great increase in the proportion of papers on



On best behaviour — green-backed heron with speared fish. (Reproduced from a cover of *Behavioural Ecology*.)

the subject appearing in ethological journals such as *Animal Behaviour*. Second, *Behavioral Ecology* is broader than *Behavioral Ecology and Sociobiology* both in format and scope. The new journal will publish reviews and scientific correspondence in addition to original papers. Details of the scope published in the society newsletter point out a use of behavioural ecology both in the sense defined above and in its other sense, the effect of behavioural processes on populations and communities. Taxonomic coverage includes animals "from invertebrates to humans" as well as plant studies related to the subject area, such as pollination biology and sexual selection. Surprisingly, the full broadness of scope is not (but should be) repeated in the most important place — the "instructions to authors" section of the two issues so far published.

The two issues I was sent make up a small sample on which to evaluate a journal. But the papers that have appeared so far are generally of a high calibre, as might be expected from the international range of editors and editorial board members (the strong taxonomic bias (avian) in the two issues will hopefully balance out with future issues).

Behavioral Ecology will attract the attention of library browsers with the alluring cover photograph on each issue. It will certainly attract the attention of personal subscribers: behavioural ecology at a low, low price. □

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Defining the common ground

Michael B. Usher

Ecological Economics: The Journal of the International Society for Ecological Economics. Editor-in-chief R. Costanza. Elsevier. 6/yr. US and Canada \$265, elsewhere Dfl. 440.

ECONOMICS has been a part of many applied environmental degree courses, such as forestry, for the past 30 years or more, but it is a subject that is missing from most ecology courses. Why is this? Is it that ecology as a science has divorced itself from the social sciences, or is there really so little common ground? Many scientific advances have been achieved by the synergistic interactions between two disciplines; can ecology and economics (both incidentally derived from the same Greek word) have such a future?

Ecological Economics was selected as a title in preference to *Economic Ecology* or *Ecology and Economics*, but it is clear that the field needs definition. Paul Ehrlich in the first issue quotes a survey in which graduate economics students did not mention ecology at all in their lists of out-of-subject reading. We do not know whether ecology graduate students treat economics similarly. Because economic decisions often have ecological implications, should this new subject of ecological economics be included in the training of both economists and ecologists?

The journal has an editorial team of 45, with membership from the United States (44 per cent), nine European nations (38 per cent) and five other nations (18 per cent). The papers, however, are more strongly biased towards the United States with 65 per cent of the authors coming from there, 22 per cent from six European nations and 13 per cent from five other nations. Do these figures tell us anything about the world distribution of research and expertise in ecological economics, about the activities of the editorial board, or simply about the writing habits of the researchers?

The journal is going to have to steer a difficult path between environmental and ecological economics. Papers on chemical emissions, environmental accounting, water quality, nonrenewable resources and energy resources, have the wider environmental perspective. Other papers focusing on wetlands, nitrogen and agricultural land, tropical agriculture, and the preservation of species, crop varieties and genetic diversity, have a more clearly defined ecological per-

spective. I welcome such papers because they provide an insight into an area of applied ecology that has too frequently been neglected. The editorial team must ensure that they guide the journal into what is truly ecological economics, avoiding its becoming another venue for publication of papers in environmental economics. Sustainability, lying on the boarder line of ecological and environmental thought, is clearly a subject well worth covering. A stringent editorial policy will be needed if this new journal is to develop and guide the field of ecological economics, as well as demonstrating its importance and excitement to students of ecology and economics alike. □

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Microbes at work

W. Bernard Betts

Biodegradation. Editors-in-chief R. S. Hanson, T. Leisinger, B. E. Rittmann, A. H. Stouthamer and W. Verstraete. Kluwer. 4/yr. Dfl. 340, £115.50, \$173 (institutional); Dfl. 190, £64.50, \$99.50 (personal)

BIODEGRADATION is a recent (1990) and timely offspring of Kluwer's *Antonie van Leeuwenhoek*, publishing original research papers and invited reviews on the detoxification, recycling, amelioration or

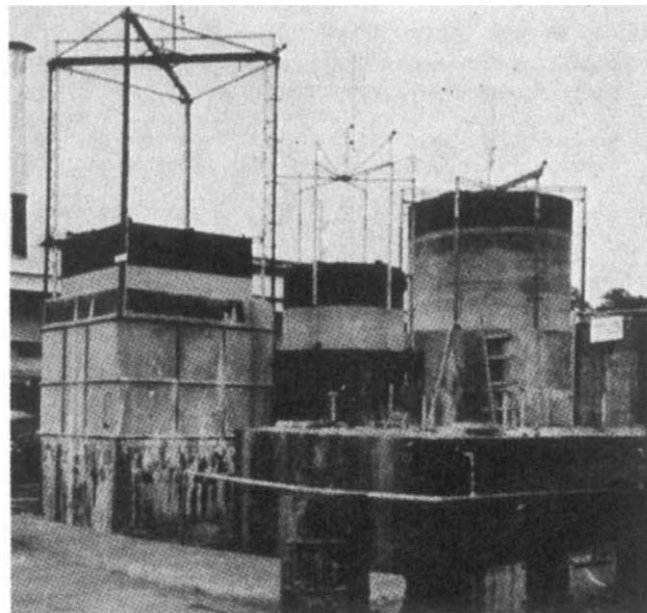
treatment of waste materials and pollutants by naturally occurring microbes (including associations) or recombinant microorganisms. The scope is broad and includes fundamental biodegradation biochemistry, physiology and genetics, modelling and scale-up of biotreatment systems for specific applications, and the development of international standards encompassing economic and legal aspects of waste treatment.

The editorial board looks impressive, comprising international experts from well-selected and important areas of biodegradation research. Indeed, the air of quality carries through, and papers published so far have been of a very high standard. The first special issue on the physiology of biodegradative microorganisms (guest editor Colin Ratledge) was an especially interesting combination of reviews examining some of the fundamental research supporting the more applied aspects of biodegradation. I look forward to special issues appearing in subsequent volumes, although the requisitioning of two issues for this purpose seems rather extravagant and leaves room for around only 14 research papers per year at current publication rates.

The journal is bright and attractive, with a large-format double-column style. Clarity is excellent and an unusual and distinctive cover logo completes the impression of good quality scientific modernism.

As the editorial in the first issue emphasizes, biodegradation is an important discipline. Much basic research is still needed to exploit the ability of microbes to degrade both recalcitrant

and xenobiotic compounds. In addition, bioremediation systems for the wide range of potential pollution problems are far from established. The journal should provide a specialized forum for the publication of research dedicated to this expanding area, and will perhaps assist authors to publish their results more quickly; the field is currently served by many of the nonspecialist journals and long delays are often involved before articles appear in print. But do not expect to have your paper published so rapidly, as the low number of annual issues and the emphasis on detailed,



Bio-gas plants were developed in Germany during the Second World War. This large plant in Manila converts the manure of 7,500 pigs into gas. (From *Paper Heroes. Appropriate Technology: Panacea or Pipe Dream?* by Witold Rybczynski. Published in paperback by Penguin, price \$9.95.)

time-consuming refereeing could cause disappointment.

Of course, research in the field can only benefit from the emphasis on quality in the long term — the editors' aim in this respect will, I hope, not suffer from 'enhanced degradation' when attracting manuscripts.

At a time when new journals often induce yawns of indifference I can for

once express a sight of relief: *Biodegradation* not only fills a definite niche but does it admirably. I look forward to an expanding number of annual issues as the journal becomes established and accepted. □

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Causes and cures

Michael L. Berrow

Land Degradation and Rehabilitation. Editor-in-chief C. J. Barrow. Wiley. 4/yr. \$150 (institutional); \$112.50, £63.75 (personal).

THERE is now much concern about the worldwide degradation of land and the accompanying reduction in ecosystem diversity. We are all made increasingly aware of problems such as desertification, erosion, deforestation, storms and flooding that affect many developing countries, whereas the effects of atmospheric pollution, mining, urbanization, sewage disposal, industrial effluents and acid rain in the more industrialized countries confront us daily. It is opportune, therefore, that a new journal addressing these serious problems in a global context has been launched.

The first issue of *Land Degradation and Rehabilitation* appeared in July 1989. Its aims and scope embrace a wide range of topics, including environments, processes, causes, perception, impacts and management, related to land degradation. The interdisciplinary and international content is well illustrated by the first six issues, which include informative contributions from the six continents. These cover topics as diverse

as soil erosion by wind and water, flooding, deforestation, compaction, salinization, effects of mining activities, revegetation using grasses or trees, remote sensing applied to derelict land, soil degradation, soil conservation, sustainable agriculture, rural development, climate change, economic development and population-resource projections. Each issue contains related book reviews.

There is a good balance of papers dealing with the problems of developed and developing countries, with about one-third of the first 34 papers reporting work in Africa and China and another third work in Europe and North America. A strength of the journal is that, as well as seeking to identify and quantify causes, it emphasizes measures to avoid, control or manage the harmful effects of degradation.

The editors stress that good data are essential for policy formulation and decision making. A plea is made for the use of statistical design in experimentation and for a considerable improvement in the quality of environmental data, even allowing for the many difficulties that arise in carrying out field research in developing countries. Judging by the quality of papers in the early issues, this challenge is being met. □

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Also submitted for review

The following is a list of journals that were eligible for review but that for one reason or another are not covered in the preceding pages. The list does not include journals sent to us that had not published enough titles to be considered.

Accounts and Rapid Communications in Synthetic Organic Chemistry (Georg Thieme Verlag)
Advances in Physiology Education (The American Physiological Society)
Biotechnology Education (Pergamon)
Blood Coagulation and Fibrinolysis (Rapid Communications of Oxford)
Chemoecology (Georg Thieme Verlag)
Chemtracts: Biochemistry and Molecular Biology (Data Trace Chemistry Publishers)
Comments on Developmental Neurobiology (Bell and Bain)
Current Opinion in Biotechnology (Current Biology)
Current Opinion in Lipidology (Current Science)
European Journal of Applied Mathematics (Cambridge University Press)
European Journal of Cancer (Pergamon)
Expert Systems with Applications (Pergamon)
Immunology and Infectious Diseases (Rapid Communications of Oxford)
International Journal of Modern Physics C (World Scientific)
Journal of Educational and Psychological Consultation (Erlbaum)
Journal of Electronic Testing: Theory and Applications (Kluwer)
Journal of Wilderness Medicine (Chapman and Hall Medical)
Land Management and Environmental Law Report (SLE Publications)
Language Acquisition: A Journal of Developmental Linguistics (Erlbaum)
Lithium (Churchill Livingstone)
Mathematics Review (Philip Allan)
Multidimensional Systems and Signal Processing (Kluwer)
Pesticide Outlook (Royal School of Chemistry)
Platelets (Churchill Livingstone)
Polar and Glaciological Abstracts (Cambridge University Press)
Psychological Inquiry (Erlbaum)
Receptor (Humana)
Restorative Neurology and Neuroscience (Elsevier)
Scientific Drilling (Springer International)
Structural Optimization (Springer)

Autumn Books

Nature's next review supplement will be Autumn Books, which will appear in the 28 November issue.



Massive land erosion in Ethiopia caused by deforestation.

PLEASE follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers who work in other fields and those for whom English is a second language, and write clearly and simply, avoiding unnecessary technical terminology. Space in the journal is limited, making competition for publication severe. Brevity is highly valued. One printed page of *Nature*, without interruptions of the text, contains about 1,300 words.

Manuscripts are selected for publication according to editorial assessment of their suitability and reports from independent referees. They can be sent to London or Washington and should be addressed to the Editor. Manuscripts may be dealt with in either office, depending on the subject matter, and will where necessary be sent between offices by overnight courier. All manuscripts are acknowledged on receipt but fewer than half are sent for review. Those that are not reviewed are returned as rapidly as possible so that they may be submitted elsewhere without delay. Contributors may suggest reviewers; limited requests for the exclusion of specific reviewers are usually heeded. Manuscripts are usually sent to two or three reviewers, who are chosen for their expertise rather than their geographical location. Manuscripts accepted for publication are typeset from the London office.

Nature requests authors to deposit sequence and crystallographic data in the databases that exist for this purpose, and to mention availability of these data.

Once a manuscript is accepted for publication, contributors will receive galley proofs in about 4 weeks. *Nature's* staff will edit manuscripts with a view to brevity and clarity, so contributors should check their proofs carefully. Manuscripts are generally published 2–3 weeks after receipt of corrected proofs. *Nature* does not exact page charges. Contributors receive a reprint order form with their proofs; reprint orders are processed after the manuscript is published and payment received.

Categories of paper

Review articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text (not including figure legends) or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles start with a heading of 50–80 words written to advertise their content in general terms, to which editors will pay particular attention. The heading does not usually contain numbers, abbreviations or measurements. The introduction to the study is contained in the first two or three paragraphs of the article, which also briefly summarize its results and implications. Articles have fewer than 50 references and may contain a few subheadings of two or three words.

Letters are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should have 1,000 or fewer words of text and four or fewer display items. The first paragraph describes, in not more than 150 words and without the use of abbreviations, the background, rationale and chief conclusions of the study for the particular benefit of non-specialist readers. Letters do not have subheadings and contain fewer than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned but suggestions can be made to the commentary editor in the form of a one-page synopsis. Commentaries are normally between one and four pages of *Nature*.

News and Views articles inform non-specialist readers about new scientific advances, sometimes in the form of a conference report. Most are commissioned but proposals can be made in advance to the

News and Views editor.

Scientific Correspondence is for discussion of topical scientific matters, including those published in *Nature*, and for miscellaneous contributions. Priority is given to letters of fewer than 500 words and 5 references.

Preparation of manuscripts

All manuscripts should be typed, double-spaced, on one side of the paper only. An original and three copies are required, each accompanied by artwork. If photographs are included, four sets of originals are required; for line drawings, one set of originals and three good-quality photocopies are acceptable. Reference lists, figure legends and tables should all be on separate sheets, all of which should be double-spaced and numbered. Relevant manuscripts in press or submitted for publication elsewhere should be included with each copy of a submitted manuscript, and clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their manuscript numbers.

Titles say what the paper is about with the minimum of technical terminology and in fewer than 80 characters in the case of Articles and Letters. Active verbs, numerical values, abbreviations and punctuation are to be avoided. Titles should contain one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name and, when known, the manuscript number. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are to be avoided and are permitted only if the parts are closely related, either experimentally or logically. Unlettered originals of photographs should be provided. Original artwork is returned when a manuscript cannot be published.

Protein/nucleotide sequences should ideally be in the three-letter and not the single-letter code for amino acids. One column width of *Nature* can accommodate 20 amino acids or 60 base pairs. Numbering of sequences should be in the left-hand margin only, with a single space between rows.

Suggestions for cover illustrations, with captions and labelled with the manuscript number, are welcome.

Colour artwork. A charge of £500 per page is made as a contribution towards the cost of reproducing colour figures. Inability to pay these costs will not prevent the publication of essential colour figures if the circumstances are explained. Proofs of colour artwork may be sent to contributors under separate cover from their galley proofs.

Figure legends should not exceed 300 words and ideally should be shorter. The figure is described first, then, briefly, the method. Reference to a method published elsewhere is preferable to a full description. Methods are not described in the text.

References are numbered sequentially as they appear in the text, followed by those in tables and finally by those in figure legends. Only papers published or in the press are numbered and included in the reference list. All other forms of reference, including unrefereed abstracts, should be cited in the text as a personal communication, manuscript submitted or in preparation. Text is not included in reference lists. References are abbreviated according to the *World List of Scientific Periodicals* (Butterworths, London, 1963–65). The first and last page numbers are included; reference to books should include publisher, place and date.

Abbreviations, symbols, units and greek letters should be identified the first time they are used. Acronyms should be avoided whenever possible and, if used, defined. Footnotes are not used except for changed addresses.

Acknowledgements are brief; grant and contribution numbers are not allowed.

Submission. Manuscripts can be sent to the Editor at 4 Little Essex Street, London WC2R 3LF, UK or at 1137 National Press Building, Washington, DC 20045, USA. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value \$5' to prevent the imposition of import duty and value-added tax (VAT). □